Performance evaluation in shipboard test of the BIO-UV ballast water management system BIO-SEA

BIO-UV
Report
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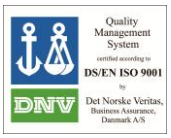	

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Performance evaluation in shipboard test of the BIO-UV ballast water management system BIO-SEA

Prepared for BIO-UV

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MOZART in Shanghai, China

Project No	11812218
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Abbreviations

Abbreviation	Description
AVG	Average
BV	Bureau Veritas
BWMS	Ballast water management system
CFU	Colony-forming units
CMFDA	Chloromethylfluorescein diacetate
DOC	Dissolved organic carbon
DWT	Deadweight tonnage
FDA	Fluorescein diacetate
FR	Field replicate
IMO	International Maritime Organization
MEPC	Marine Environment Protection Committee
MPN	Most probable number
NTU	Nephelometric turbidity unit
POC	Particulate organic carbon
PSU	Practical salinity unit
QAPP	Quality assurance project plan
QMP	Quality management plan
SOP	Standard operating procedure
STD	Standard deviation
TSS	Total suspended solids
UVT	UV transmittance

1 Executive summary and conclusion

DHI provides independent performance evaluation testing to manufacturers of ballast water management systems. DHI's quality assurance project plan is consistent with the requirements of the International Convention for the Control and Management of Ships Ballast Water and Sediments.

From June 2012 through January 2013, DHI conducted shipboard tests of the BIO-UV ballast water management system BIO-SEA in accordance with DHI's certification by Lloyd's Register verified by Bureau Veritas. The ability of BIO-SEA to (a) successfully treat ballast water without interruption and (b) meet IMO D-2 discharge standards was evaluated during a series of three valid test cycles.

The first test cycle #1 was conducted in the Port of Shanghai, China, in June 2012 where the source water was fresh (< 3 practical salinity units, PSU) and the water temperature was 26°C. Discharge operation was due to security reasons performed by the vessels crew without the possibility of a second treatment and sampling of discharge water. The first test was thus considered unsuccessful and three further test cycles had to be performed. The second test cycle was performed in the port of Tanjung Pelepas, Malaysia, in December 2012 at a salinity of >30 PSU and water temperature of 30°C. The two remaining test cycles were conducted in Australia in January 2013 in the port of Brisbane and the port of Melbourne, respectively, at salinities of 34-37 PSU and water temperatures of 22-27°C.

The average densities of viable organisms in the ≥50 µm size class in the inlet water varied from approx. 5,000 to approx. 175,000 organisms/m^3. For the size class ≥10 and <50 µm, the average densities in the inlet water in the three test cycles (#2, #3 and #4) were 85, 503 and 783 organisms/mL. In test cycle #2, the average density of the organisms in the size class ≥10 and <50 µm in the inlet water was 85 organisms/mL which is below the required density of 100 organims/mL for a valid test cycle. To further examine the density of smaller planktonic organisms in the inlet water between 6-9 µm in the minimum dimension were enumerated. The density of organisms between 6-9 µm in the inlet water was 33 organisms/mL which implies that the total density (size class 6-10 µm and 10 to <50 µm) was 118 organisms/mL. This total density corresponded well with the density of autrotrophic organisms determined by a re-growth assay, which included all photosynthetic algae regardless of size. The density of autrotrophic organisms determined by the re-growth assay was >160 organisms/mL. Although the inlet water density of organisms/mL in the size class ≥10 and <50 µm was below the validity criterion in the IMO G8 guidelines, the total density of organisms was sufficient for the performance evaluation of the BWMS and thus test cycle #2 is considered valid.

The contents of *E. coli* were generally low and enterococci were hardly detected. For shipboard testing, there are no requirements in the IMO G8 guidelines in relation to the density of bacteria in the inlet water.

In the treated discharge water, the numbers of viable organisms in the ≥50 µm size class were respectively 1.5, 2.1 and 2.6 organisms/m^3 in the three test cycles #2, #3 and #4. The numbers of viable organisms in the ≥10 and <50 µm size class in the treated discharge water were 0.7, 0.4 and 1.7 organisms/mL in test cycles #2, #3 and #4 when determined by direct counting after vital staining with CMFDA/FDA. Densities of viable algae in the treated discharge water were determined to be <0.18,0.45 and 0.28 organisms/mL when viability was determined by use of the re-growth assay. In the treated discharge water, the average contents of *E. coli* and enterococci were below the detection limit in all three test cycles. *Vibrio cholerae* was not identified in any of the test cycles.

The BIO-SEA functioned properly during all three test cycles #2, #3 and #4 and effectively reduced live organism densities. Live organisms in the size classes defined in the IMO G8 guidelines (MEPC.174(58) (G8)) were discharged at densities below the IMO D-2 standard.

2 Introduction

The objective of this project was to conduct a shipboard test of the BIO-UV ballast water management system BIO-SEA in accordance with the guidance given in Resolution MEPC.174(58), Guidelines for approval of ballast water management systems (G8) (IMO, 2008), hereafter referred to as the IMO G8 guidelines.

DHI holds a certificate of compliance issued by Lloyd's Register. The acting classification society for the shipboard test of the BIO-UV ballast water management system (BWMS) BIO-SEA was Bureau Veritas (BV).

DHI has no involvement, intellectual or financial, in the mechanics, design or marketing of the BWMS whose performance has presently been evaluated. To ensure that DHI tests are uncompromised by any real or perceived individual or team bias relative to test outcomes, DHI test activities are subject to rigorous quality assurance, quality control procedures and documentation.

During the shipboard testing campaigns, a BIO-SEA ballast water treatment unit with automatic filtration and UV light reactors with nominal flow capacities of up to 1000 m^3/h was tested. The BIO-SEA installation consisted of ten UV light reactors in order to match the pump capacity of the vessels ballast pumps.

During three consecutive valid test cycles, the BIO-SEA was evaluated for its ability to: (a) successfully treat ballast water without interruption and (b) meet IMO D-2 standard at discharge.

3 Testing laboratory

DHI is an independent, international consulting and research organisation with the objectives to advance technological development and competence within the fields of water, environment and health.

The shipboard test was carried out by:

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4 Ballast water management system

A description of the BWMS BIO-SEA as provided by BIO-UV is included in the quality assurance project plan (QAPP) in Appendix C.

The four test cycles were conducted with a Filtrex (40 µm mesh) filter.

5 Experimental design

5.1 Trial periods and locations

The shipboard test was conducted on-board the CMA CGM container vessel MOZART (IMO 9280615). CMA CGM MOZART is a container ship from 2004. CMA CGM MOZART is a Bureau Veritas class general container ship registered in France. During the shipboard testing period, the CMA CGM MOZART was in regular route. The BWMS BIO-SEA was installed in the engine

room of the ship. For the shipboard testing, the ballast tank pair 7 were used for treated water and the ballast tank pair 4 was used for control water. The individual tests with the BWMS BIO-SEA were conducted as presented in Table 5.1.

Table 5.1 Details for inlet and discharge operations for shipboard test cycles. The time indicated reflects the local time.

Test cycle	Ballast-Location	Operation	Inlet	Volume and flow rate	Discharge	Volume and flow rate
BIO-SEA Test#1	Shanghai	Control	2012.06.26 10:31-11:04	580 m³ 990 m³/h	2012.06.27 Approx. 22:00	No sampling performed
		Treatment	2012.06.26 15:00-15:32	470 m³ 990 m³/h	2012.06.27 Approx. 22:00	No sampling performed
BIO-SEA Test#2	Tanjung Pelepas	Control	2012.12.05 17:20-17:55	474 m³ 908; 770; 762 m³/h	2012.12.06 23:50-0:30	425 m³ 580; 670; 661 m³/h
		Treatment	2012.12.05 20:55-22:30	1461 m³ 951; 917; 901 m³/h	2012.12.06 22:40-23:40	889 m³ 915; 900; 853 m³/h
BIO-SEA Test#3	Brisbane, Australia	Control	2013.01.19 10:25-10:56	549 m³ 806; 816; 788 m³/h	2013.01.21 09:43-10:25	485 m³ 702; 692; 685 m³/h
		Treatment	2013.01.19 11:35-12:40	913 m³ 850; 836; 844 m³/h	2013.01.21 08:38-09:18	552 m³ 842; 825; 819 m³/h
BIO-SEA Test#4	Melbourne, Australia	Control	2013.01.24 14:25-15:28	926 m³ 876; 890; 879 m³/h	2013.01.26 10:17-10:58	459 m³ 690; 681; 645 m³/h
		Treatment	2013.01.24 15:47-17:10	1.154 m³ 886; 820; 796 m³/h	2013.01.26 09:17-10:03-	637 m³ 849; 820; 823 m³/h

- **Inlet water:** The ballast operations were all performed in the described ports. Physical-chemical and biological parameters in the inlet water were considered as sufficiently stable during the ballasting, and, thus, only one set of samples and analyses was used to represent the control tank and the ballast tank
- **Discharge control water:** Stored without treatment from the time of ballasting to discharge
- **Discharge treated water:** Treated and stored from the time of ballasting to discharge

5.2 Sampling

5.2.1 Sample overview

All samples were collected by DHI staff in accordance with the description in the QAPP (Appendix C).

Table 5.2 Number of samples and sample volumes

Water type	Sample replicates	Sample volume per replicate
Inlet water	3 replicates	Organisms ≥50 µm: >1 m^3 *
		Organisms ≥10 and 50 µm: >2 L **
		Bacteria: >0.5 L **
		DOC + POC: Approx. 0.5 L **
		TSS: Approx. 0.5 L **
Control discharge water	3 replicates	Organisms ≥50 µm: >1 m^3 *
		Organisms ≥10 and 50 µm: >2 L **
		Bacteria: >0.5 L **
		DOC + POC: Approx. 0.5 L **
		TSS: Approx. 0.5 L **
Treated discharge water	3 replicates	Organisms ≥50 µm: >3 m^3 *
	3 × 3 replicates	Organisms ≥10 and 50 µm: >2 L **
	3 × 3 replicates	Bacteria: >0.5 L **
	3 replicates	DOC + POC: Approx. 0.5 L **
	3 replicates	TSS: Approx. 0.5 L **

* Samples collected by continuous flow during the entire period of intake or discharge; this continuous sampling of 3 replicates, each with a volume >3 m^3, provides the same statistical basis for counting as the sampling of 3 × 3 replicates of >1 m^3, which is recommended in the IMO G8 guidelines

** Samples collected over the period of intake or discharge (start, middle and end)

DOC Dissolved organic carbon

POC Particulate organic carbon

TSS Total suspended solids

5.2.2 Samples for enumeration of organisms ≥50 µm

Three replicates were collected by parallel continuous sampling during the entire periods of inlet and discharge. The samples were gently filtered through a net with a mesh size of 35 µm and a reservoir (cod-end) at the bottom for collecting the organisms ≥50 µm. Each replicate was transferred to 1-L glass bottles. The total volume of the filtered sample exceeded 3 m^3 per replicate for the treated discharge samples and 1 m^3 per replicate for the inlet and control discharge samples. The exact sample volume for each of the three replicates was determined by use of three flow meters that were connected to the relevant sampling ports installed in the system.

5.2.3 Samples for enumeration of organisms ≥10 µm and <50 µm

Samples (3 replicates for the inlet water, 3 replicates for the control discharge water, and 3 × 3 replicates for the treated discharge water) with a volume of at least 1 L were collected in polyethylene containers.

5.2.4 Samples for enumeration of bacteria

Samples (3 replicates for the inlet water, 3 replicates for the control discharge water, and 3 × 3 replicates for the treated discharge water) with a volume of at least 0.5 L were collected in sterile polyethylene containers.

5.2.5 Samples for DOC, POC and TSS analyses

Samples (3 replicates for the inlet water, 3 replicates for the control discharge water, and 3 replicates for the treated discharge water) of at least 0.5 L were collected in heat-sterilized blue cap bottles for analysis of DOC and POC. For TSS analysis, samples with a volume of approx. 0.5 L were collected in polyethylene containers.

5.3 Analyses

5.3.1 Analysis overview

Table 5.3 Overview of analyses and sample replicates

Replicate	Temperature	Salinity	≥ 50 µm	10-50 µm, Microscopy	10-50 µm, Re-growth	Bacteria	DOC + POC	TSS
Inlet water								
Replicate 1 (start)	1	1	Three replicates	1	1	1	1	1
Replicate 2 (mid)	2	2		2	2	2	2	2
Replicate 3 (end)	3	3		3	3	3	3	3
Control discharge water								
Replicate 1 (start)	1	1	Three replicates	1	1	1	1	1
Replicate 2 (mid)	2	2		2	2	2	2	2
Replicate 3 (end)	3	3		3	3	3	3	3
Treated discharge water								
Replicate 1-3 (start)	1	1	Three replicates	1	1-3	1-3	1	1
Replicate 4-6 (mid)	4	4		4	4-6	4-6	4	4
Replicate 7-9 (end)	7	7		7	7-9	7-9	7	7

DOC Dissolved organic carbon
POC Particulate organic carbon
TSS Total suspended solids

All analyses were carried out in accordance with the QAPP + Amendment No. 1 (Appendix C) and the relevant standard operating procedures (DHI SOPs). Samples were processed on-board the vessel within 4 hours from the time of sampling.

Table 5.4 Sample storage temperature from sampling to analysis

Sample	Storage temperature (°C)		
	From sampling to handling on board (<4h)	Storage on board (up to 48 h)	Shipment to DHI (up to 72 h)
Organisms ≥50 µm	20-25	-*	-*
Organisms ≥10 and < 50 µm (CMFDA/FDA)	20-25	5	5-15
Organisms ≥10 and < 50 µm (Re-growth)	20-25	20-25	5-15
Organisms ≥10 and < 50 µm (Lugol's solution)	20-25	5	5-15
Bacteria	20-25	-*	-*
POC, DOC, TSS	20-25	5	5-15

* No storage of samples; the analyses were initiated and completed on location

5.3.2 Organism size class ≥50 µm

The concentrations of viable organisms ≥50 µm in the samples were determined by using a stereo microscope and a counting chamber. Viable organisms were determined on the basis of observed mobility and morphology according to DHI SOP 30/1700. The viable organisms were

characterized according to broad taxonomic groups. Compliance with the IMO D-2 standard (IMO, 2004) was verified by using the direct count of viable organisms ≥50 µm.

5.3.3 Organism size class ≥10 µm and <50 µm

The polyethylene container with a volume of 2.5 L was gently turned upside down five times, after which subsamples were taken for the analyses described below.

Organisms in inlet water

The concentrations of organisms in the size class ≥10 and <50 µm were determined by inverted microscopy. Subsamples with a volume of approx. 100 mL were transferred from the inlet water samples to brown 100-mL glass bottles. The subsamples were preserved by addition of Lugol's solution to achieve 2% final concentration. The subsamples were sealed and kept in the dark at approx. 5°C during storage on-board the vessel and transported to the DHI laboratory by courier. The organisms identified in the inlet water were assumed to be viable at the time of sampling. The identification comprised detailed examination of the algal chloroplasts to confirm that the organisms had been alive and classification of the algae in major taxonomic groups.

One subsample per replicate, with a volume of approx. 10 mL, was transferred to 10-mL polyethylene tubes with screw-caps. The concentrations of viable algae were determined by use of a re-growth assay by use of the most probable number (MPN) technique. A dilution series was made for each replicate and 1 mL-aliquots containing 1 mL, 0.1 mL and 0.01 mL of the subsample were added to series of five test tubes with 5 mL of liquid medium. Blank controls containing 5 mL of liquid medium without sample were also prepared. The test tubes were sealed and kept in the dark at ambient temperature during storage on-board the vessel and transported to the DHI laboratory by courier. The fluorescence of the test tubes was determined prior to the incubation. The concentrations of viable algae in the samples were determined by measuring the fluorescence in the test tubes after 14 days of incubation under continuous light. Incubation temperatures were in the range 23-29°C dependent on the ambient temperature during ballast operation.

Organisms in discharge water

The concentrations of viable algae in the control discharge water and the treated discharge water were determined by use of the re-growth assay as described above.

Furthermore, viable organisms in the ≥10 µm and <50 µm size class were determined by vital staining with chloromethylfluorescein diacetate (CMFDA) and fluorescein diacetate (FDA) solution. Subsamples with a volume of approx. 200 mL were transferred from the control and treated discharge water samples to brown 200-mL glass bottles. The subsamples were sealed and kept in the dark at approx. 5°C during storage on-board the vessel and transported to the DHI laboratory by courier. CMFDA and FDA was added to a subsample and after incubation, the subsample was examined by use of a microscope under epifluorescence. Organisms labelled by either CMFDA or FDA were considered viable as described in DHI SOP 30/1701.

5.3.4 Bacteria

The concentrations of *E. coli* and enterococci were determined by diluting the samples with a special microplate diluent (1:1), after which the samples were distributed in a specific 96-wells test kit for either *E. coli* or enterococci (BIO-RAD, MUG/MUD kits for *E. coli* or enterococci quantification). The inoculated test kits were incubated for 36 hours at 44°C (42°C to 44.5°C), after which positive wells were used to calculate the most probable numbers.

Samples for detection of *Vibrio cholerae* were filtered through a 0.45-µm filter. The filters were stored in the dark at ambient temperature in sterile polyethylene tubes and transported to the DHI laboratory by courier. On arrival at the laboratory, the filters were submerged into alkaline saline peptone water for two selective enrichments. The cultures obtained by the enrichments were used for inoculation of agar plates. Following 24 hours of incubation at 37 ± 1°C, the morphology of the colonies on the agar plates was inspected. In case of *Vibrio cholerae* positive results, further identification is performed by Statens Serum Institute (SSI), Denmark.

5.3.5 Physical-chemical parameters

Dissolved oxygen, salinity, temperature and pH were measured by use of portable multi parameter instrument equipped with electrodes. In test cycles # 1, #3 and #4 (Table 5.1), turbidity was also measured. Measurements were conducted at regular intervals throughout the inlet and discharge operations. Turbidity was not measured in test cycle #2.

Samples for determination of organic carbon content were filtered through a 0.45-µm syringe filter. By using a TOC analyser, the TOC was determined by analysis of non-filtered samples whereas the dissolved organic carbon (DOC) was determined by analysis of filtered samples. The particulate organic carbon (POC) was calculated as the difference between TOC and DOC.

Samples for determination of total suspended solids (TSS) were filtered through a glass fibre filter, which had already been weighed in the laboratory, and the TSS was determined by weighing of filters containing sample after drying at 105°C.

6 Results

6.1 Physical-chemical parameters

For the BIO-SEA system, the physical-chemical conditions of inlet and discharge waters for test cycles are summarized in Tables 6.1 and 6.2. Onsite measurement data are also available in the data logging in Appendix A. Detailed data on TSS, POC and DOC are available in Appendix B.

Table 6.1 Average concentrations of total suspended solids (TSS), particulate organic carbon (POC) and dissolved organic carbon (DOC)

Test cycle	Water type	TSS (mg/L)	POC (mg/L)	DOC (mg/L)
BIO-SEA Test#1	Inlet water	145	1.9	2.4
	Treated water (T0)	n.d.	n.d.	n.d.
	Control discharge water	n.d.	n.d.	n.d.
	Treated discharge water	n.d.	n.d.	n.d.
BIO-SEA Test#2	Inlet water	77	1.1	0.32
	Treated water (T0)	44	n.d.	n.d.
	Control discharge water	8.3	0.54	0.52
	Treated discharge water	43	0.85	0.29
BIO-SEA Test#3	Inlet water	14	0.46	1.2
	Treated water (T0)	19	n.d.	n.d.
	Control discharge water	15	<0.1	1.3
	Treated discharge water	15	<0.1	1.2
BIO-SEA Test#4	Inlet water	17	<0.1	1.1
	Treated water (T0)	16	<0.1	n.d.
	Control discharge water	13	<0.1	0.85
	Treated discharge water	24	<0.1	0.79

n.d. Not determined

Table 6.2 Average measurements of oxygen (O_2), salinity, temperature, pH, UV transmittance (UVT) and turbidity

Test cycle	Water type	O_2 (%)	Salinity (PSU)	Tempera-ture (°C)	pH	UVT (%)	Turbidity (NTU)
BIO-SEA Test#1	Inlet water	95	0.2	26.4	7.5	49	83
	Control discharge water	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Treated discharge water	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BIO-SEA Test#2	Inlet water	64	31	29.9	7.8	83	n.d.
	Control discharge water	75.8	31	29.9	7.8	92	n.d.
	Treated discharge water	87.1	30	30.5	7.7	92	n.d.
BIO-SEA Test#3	Inlet water	81.3	36.8	27.6	8.0	92	6.8
	Control discharge water	72.9	36.4	25.3	7.9	94	3.1
	Treated discharge water	81.3	36.7	26.9	7.9	98	3.3
BIO-SEA Test#4	Inlet water	77.8	34.3	22.0	7.9	87	5.5
	Control discharge water	75.7	34.4	19.5	7.8	90	5.5
	Treated discharge water	73.4	34.1	21.3	7.8	89	3.9 (15.2*)

PSU Practical salinity units
NTU Nephelometric turbidity units
UVT UV transmittance
* One replicate (FR3) was exstremely high (15.2)
n.d. Not determined

6.2 Biological parameters

The densities of live organisms in the inlet and control discharge water were in accordance with the IMO G8 guidelines (IMO, 2008) in all test cycles. Detailed data from the biological efficacy analyses are available in Appendix B.

6.2.1 Organism size class ≥50 µm

The average densities of viable organisms in the ≥50 µm size class varied from approx. 5,000 to 175,000 organisms/m^3 in the inlet water and from approx. 6,500 to 48,000 organisms/m^3 in the control discharge water (Table 6.3).

Table 6.3 Total sample volumes and average concentrations of viable organisms in the size class ≥50 µm. Detailed data and individual sample volumes are provided in Appendix B.

Test cycle	Inlet water		Control discharge water		Treated discharge water	
	Total sam-ple volume (m^3)	Organ-isms/m^3	Total sam-ple volume (m^3)	Organ-isms/m^3	Total sample volume (m^3)	Organ-isms/m^3
BIO-SEA Test#1	3.570	12,694	n.d.	n.d.	n.d.	n.d.
BIO-SEA Test#2	3.244	25,997	3.094	13,997	9.451	1.6
BIO-SEA Test#3	3.162	5,424	3.229	6,648	9.601	2.1
BIO-SEA Test#4	3.958	175,047	3.448	48,088	9.854	2.6
IMO G8	≥3	≥100	≥3	≥10	≥9	<10

In the treated discharge water, the numbers of viable organisms in the ≥50 µm size class varied from 1.6 to 2.6 organisms/m^3.

6.2.2 Organism size class ≥10 and <50 µm

For the size class ≥10 and <50 µm, the average densities in the inlet water in the three test cycles (#2, #3 and #4) were 85, 503 and 783 organisms/mL. In test cycle #2, the average density of the organisms in the size class ≥10 and <50 µm in the inlet water was 85 organisms/mL which is below the required density of 100 organims/mL for a valid test cycle. To further examine the density of smaller planktonic organisms in the inlet water, organisms between 6-9 µm in the minimum dimension were enumerated. The density of organisms between 6-9 µm in the inlet water was 33 organisms/mL which implies that the total density (size class 6-10 µm and 10 to <50 µm) was 118 organisms/mL (Table 6.4). This total density corresponded well with the density of autotrophic organisms determined by the re-growth assay, which included all autotrophic organisms regardless of size. The density of organisms determined by the re-growth assay was >160 organisms/mL. Although the inlet water density of organisms/mL in the size class ≥10 and <50 µm was below the validity criterion in the IMO G8 guidelines, the total density of organisms was sufficient for the performance evaluation of the BWMS and thus test cycle #2 is considered valid.

Evaluation of viable organisms in the ≥10 µm and <50 µm size class by vital staining with chloromethylfluorescein diacetate (CMFDA) and fluorescein diacetate (FDA) solution showed a considerable decrease in the number of viable organisms. In the treated discharge water, the average numbers of viable organisms in the ≥10 µm and <50 µm size class varied from 0.7 to 1.7 organisms/mL. For the control discharge water samples, the average densities of organisms in the size class ≥10 and <50 µm were 10.2, 239 and 209 organisms/mL in test cycles #2, #3 and #4 (Table 6.4).

The re-growth of algae in the treated discharge water was below the detection limit of the assay of 0.18 organisms/mL in test cycle #2. In test cycles #3 and #4, the average densities of viable algae determined by the re-growth assay were 0.45 and 0.28 organisms/mL, respectively (Table 6.4). Detailed data are provided in Appendix B.

Table 6.4 Average concentrations of viable organisms in the size class ≥10 µm and <50 µm. *, number in parenthesis represents viable organisms with the size 6-9 µm. Detailed data are provided in Appendix B.

Test cycle	Water type	Microscopy (organisms/mL)	Re-growth (organisms/mL)
BIO-SEA Test#1	Inlet water	158	>160
	Control discharge water	n.d.	n.d.
	Treated discharge water	n.d.	n.d.
BIO-SEA Test#2	Inlet water	85 (+35)*	>160
	Control discharge water	10.2	>153
	Treated discharge water	0.70	<0.18
BIO-SEA Test#3	Inlet water	503	>160
	Control discharge water	239	>160
	Treated discharge water	0.33	0.45
BIO-SEA Test#4	Inlet water	783	>160
	Control discharge water	209	>160
	Treated discharge water	1.7	0.28
IMO G8	Inlet water	≥100	≥100
	Control discharge water	≥10	≥10
	Treated discharge water	<10	<10

Table 6.5 Algal species identified in inlet water and their capability for growth under the conditions applied in the re-growth assay.

Groups and species	BIO-SEA Test #2	BIO-SEA Test #3	BIO-SEA Test #4	Capable to grow in re-growth as-say
Bacillariophyceae				
Chaetoceros affinis		X	X	X
Chaetoceros compressus			X	
Chaetoceros decipiens		X		X
Cyclotella stelligera			X	
Leptocylindrus minimus			X	X
Nitzschia longissima/Cylindrotheca	X	X		X
Paralia sulcata			X	
Phaeodactylum tricornutum	X			X
Pinnularia sp.		X		X
Porosira glacialis		X		X
Rhizosolenia delicatula		X		
Rhizosolenia setigera		X		
Skeletonema costatum	X	X	X	X
Thalassiosira augusta-lineata		X	X	X
Thalassiosira baltica		X	X	X
Dictyocophyceae				
Dictyocha speculum			X	
Dinophyceae				
Ceratium fusus		X		X
Glenodinium berghii			X	
Heterocapsa triquetra			X	X
Oblea rotunde			X	
Peridinium umbonatum		X		
Prorocentrum lima		X		
Prorocentrum micans		X		X
Protoperidinium sp.		X		
Pyrocystis fusiformis		X		
Euglenophyceae				
Trachelomonas plantonica			X	

Growth under the conditions applied in the re-growth assay has been confirmed for 100%; 63% and 54%, respectively, of the algal species identified in the inlet water in test cycles #2, #3 and #4 (Table 6.5).

UV irradiation causes damage of the DNA in the cells and it may take several days before the cell membrane is disrupted and the enzyme activity stops (Stehouwer et al., 2010, Liltved et al., 2011; Liebich et al., 2012; Martinez et al., 2013). Enumeration of algae by use of the re-growth assay is directly related to growth over a certain time period. The ability of algal species to grow is the meaningful definition of viability in an evaluation, of which the target is to determine the efficiency of treatment to reduce the potential of species in ballast water to proliferate and survive in the natural environment. For UV treatment systems, the re-growth assay is considered the best available methodology for evaluation of viable algae. The re-growth assay analyses is not limited to the ≥10 and <50 µm size class; on the contrary, this parameter include planktonic algae without reference to size.

6.2.3 Bacteria

For shipboard testing, there are no requirements in the IMO G8 guidelines in relation to the density of bacteria in the inlet water or the control discharge water.

Table 6.6 Average bacterial concentrations. Detailed data are provided in Appendix B.

Test cycle	Water type	*E. coli* (CFU/100 mL)	Enterococci (CFU/100 mL)	*Vibrio cholerae* (CFU/100 mL)
BIO-SEA Test#1	Inlet water	<10	<10	<1
	Control discharge water	n.d.	n.d.	n.d.
	Treated discharge water	n.d.	n.d.	n.d.
BIO-SEA Test#2	Inlet water	43	<10	<1
	Control discharge water	<10	<10	<1
	Treated discharge water	<10	<10	<1
BIO-SEA Test#3	Inlet water	14	<10	<1
	Control discharge water	<10	<10	<1
	Treated discharge water	<10	<10	<1
BIO-SEA Test#4	Inlet water	<10	<10	<1
	Control discharge water	<10	<10	<1
	Treated discharge water	<10	<10	<1
IMO G8	Inlet water	-	-	-
	Control discharge water	-	-	-
	Treated discharge water	<250	<100	<1

CFU Colony-forming units

The contents of *E. coli* and enterococci in the inlet water and control discharge water were generally low compared with the IMO D-2 threshold values. In the treated water, the contents of *E. coli* and enterococci were below the detection limit in all three test cycles. *Vibrio cholerae* was not identified in any of the test cycles.

7 References

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APPENDIX A

Data logging from the shipboard testing with BIO-SEA

Table A.1.1 Test cycle data logging; treated water

Subject	Results	Comments
Test cycle No.	#1	
Client treatment system	BIO-SEA	Filtrex 40µm, 10 UV lamps
Salinity (PSU)	0.2	
Ballast tank No.	5, starboard side	
Location for intake	Shanghai	
Date and time intake start	2012.06.26 15:00	
Date and time intake stop	2012.06.26 15.32	
Flow during intake	990 m^3/h	
Treated volume during intake	470 m^3	
Location for discharge	Ningbo	Discharge was done by the crew for security reasons, without warning the test team
Date and time discharge start	2012.06.27 ≈ 22:00	
Date and time discharge stop	2012.06.27 ≈ 23:00	
Flow during discharge	n.d.	
Treated volume during discharge	n.d.	

Table A.1.2 Test cycle data logging; control water

Subject	Results	Comments
Test cycle No.	#1	
Salinity (PSU)	0.2	
Ballast tank No.	5, port side	
Date and time intake start	2012.06.26 10:31	
Date and time intake stop	2012.06.26 11.04	
Flow during intake	990 m^3/h	
Volume during intake	580 m^3	
Date and time discharge start	2012.06.27 ≈ 22:00	
Date and time discharge stop	2012.06.27 ≈ 23:00	
Flow during discharge	n.d.	Discharge was done by the crew for security reasons, without warning the test team
Volume during discharge	n.d.	

Table A.1.3 Test cycle data logging; onsite measurements

Water type	Dissolved oxygen (mg/L)	Salinity (PSU)	Tempera-ture (°C)	pH	Turbidity (NTU)	UVT (%)
Inlet water (control)	4.0 (±0.04)	0.2 (±0.01)	26.7 (±0.06)	7.5 (±0.01)	83 (±7.0)	49 (±1.5)
Inlet water (treated)	4.0 (±0.10)	0.2 (±0.02)	26.4 (±0.05)	7.5 (±0.01)	96 (±5.7)	44 (±0.58)
Control discharge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Treated discharge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* Standard deviations in parentheses
PSU Practical salinity units
NTU Nephelometric turbidity unit
UVT UV transmittance
n.d. Not determined

Table A.2.1 Test cycle data logging; treated water

Subject	Results	Comments
Test cycle No.	#2	
Client treatment system	BIO-SEA	Filtrex 40µm, 10 UV lamps
Salinity (PSU)	30.4	
Ballast tank No.	7-wings, port + starboard side	
Location for intake	Tanjung Pelepas	
Date and time intake start	2012.12.05 20:55	
Date and time intake stop	2012.12.05 22.30	
Flow during intake (start; mid; end)	951; 917; 901 m^3/h	
Treated volume during intake	1461 m^3	
Location for discharge	At anchorage outside Tanjung Pelepas. GPS point: 01°15,70N; 103°33,69E	During de-ballast, BIO-SEA was run with 9 UV lamps
Date and time discharge start	2012.12.06 22:40	
Date and time discharge stop	2012.12.06 23:40	
Flow during discharge (start; mid; end)	915; 900; 853 m^3/h	
Treated volume during discharge	889 m^3	

Table A.2.2 Test cycle data logging; control water

Subject	Results	Comments
Test cycle No.	#2	
Salinity (PSU)	30.7	
Ballast tank No.	4-wings, port + starboard side	
Date and time intake start	2012.12.05 17:20	
Date and time intake stop	2012.12.05 17:55	
Flow during intake (start; mid; end)	908; 770; 762 m^3/h	
Volume during intake	474 m^3	
Date and time discharge start	2012.12.06 23:50	
Date and time discharge stop	2012.12.07 00:30	
Flow during discharge (start; mid; end)	580; 670; 661 m^3/h	
Volume during discharge	425 m^3	

Table A.2.3 Test cycle data logging; onsite measurements

Water type	Dissolved oxygen (%)	Salinity (PSU)	Tempera-ture (°C)	pH	Turbidity (NTU)	UVT (%)
Inlet water (control)	64 (±0.58)	31 (±0.3)	29.9 (-)	7.8 (-)	n.d	84 (±2.6)
Inlet water (treated)	64 (±1.2)	30 (±0.1)	30 (±0.1)	7.8 (-)	n.d	83 (±2.0)
Control discharge	76 (±2.1)	31 (±0.1)	29.9 (±0.1)	7.8 (-)	n.d	92 (±0.3)
Treated discharge	83 (±5.7)	30 (±0.4)	30.5 (±0.2)	7.7 (-)	n.d	92 (±0.3)

* Standard deviations in parentheses
PSU Practical salinity units
NTU Nephelometric turbidity unit
UVT UV transmittance
n.d. Not determined

Table A.3.1 Test cycle data logging; treated water

Subject	Results	Comments
Test cycle No.	#3	
Client treatment system	BIO-SEA	Filtrex 40µm, 10 UV lamps
Salinity (PSU)	36.7	
Ballast tank No.	7-wings, port + starboard side	
Location for intake	Brisbane, Australia	During ballast BIO,-SEA was run with 9 UV reactors on
Date and time intake start	2013.01.19 11:35	
Date and time intake stop	2013.01.19 12:40	
Flow during intake (start; mid; end)	850; 836; 844 m^3/h	
Treated volume during intake	913 m^3	
Location for discharge	Sydney, Australia	During de-ballast, BIO-SEA was run with 9 reactors on
Date and time discharge start	2013.01.21 08:38	
Date and time discharge stop	2013.01.21 09:18	
Flow during discharge (start; mid; end)	842; 825; 819 m^3/h	
Treated volume during discharge	552 m^3	

Table A.3.2 Test cycle data logging; control water

Subject	Results	Comments
Test cycle No.	#3	
Salinity (PSU)	36.7	
Ballast tank No.	4-wings, port + starboard side	
Date and time intake start	2013.01.19 10:25	
Date and time intake stop	2013.01.19 10:56	
Flow during intake (start; mid; end)	806; 816; 788 m^3/h	
Volume during intake	549 m^3	
Date and time discharge start	2013.01.21 09:43	
Date and time discharge stop	2013.01.21 10:25	
Flow during discharge (start; mid; end)	702; 692; 685 m^3/h	
Volume during discharge	485 m^3	

Table A.3.3 Test cycle data logging; onsite measurements

Water type	Dissolved oxygen (%)	Salinity (PSU)	Tempera-ture (°C)	pH	Turbidity (NTU)	UVT (%)
Inlet water (control)	81 (±1.7)	36.7 (-)	27.6 (±0.2)	8.0 (-)	6.8 (-)	92 (±1.5)
Inlet water (treated)	82 (±1.0)	36.8 (-)	27.9 (±0.2)	8.0 (-)	6.8 (-)	92 (±1.1)
Control discharge	73 (±1.1)	36.4 (±2.7)	25 (±0.2)	7.9 (-)	3.1 (±0.3)	94 (±4.5)
Treated discharge	81 (±4.0)	36.7 (±0.1)	27 (±0.1)	7.9 (-)	3.3 (±0.3)	98 (±1.5)

* Standard deviations in parentheses
PSU Practical salinity units
NTU Nephelometric turbidity unit
UVT UV transmittance

Table A.4.1 Test cycle data logging; treated water

Subject	Results	Comments
Test cycle No.	#4	
Client treatment system	BIO-SEA	Filtrex 40µm, 10 UV lamps
Salinity (PSU)	33.8	
Ballast tank No.	7-wings, port + starboard side	
Location for intake	Melbourne, Australia	During ballast, BIO-SEA was run with 9 UV reactors on. At the end of the ballast operation, BIO-SEA was run with only 8 UV reactors on.
Date and time intake start	2013.01.24 15:47	
Date and time intake stop	2013.01.24 17:10	
Flow during intake (start; mid; end)	886; 820; 796 m^3/h	
Treated volume during intake	Approx. 1.154 m^3	
Location for discharge	At open sea. GPS point: 36°51,0S; 139°08,1E, Australia	During de-ballast, BIO-SEA was run with 9 reactors on
Date and time discharge start	2013.01.26 09:17	
Date and time discharge stop	2013.01.26 10:03	
Flow during discharge (start; mid; end)	849; 820; 823 m^3/h	
Treated volume during discharge	Approx. 637 m^3	

Table A.4.2 Test cycle data logging; control water

Subject	Results	Comments
Test cycle No.	#4	
Salinity (PSU)	34.4	
Ballast tank No.	4-wings, port + starboard side	
Date and time intake start	2013.01.24 14:25	
Date and time intake stop	2013.01.24 15:28	
Flow during intake (start; mid; end)	876; 890; 879 m^3/h	
Volume during intake	926 m^3	
Date and time discharge start	2013.01.26 10:17	
Date and time discharge stop	2013.01.26 10:58	
Flow during discharge (start; mid; end)	690; 681; 645 m^3/h	
Volume during discharge	459 m^3	

Table A.4.3 Test cycle data logging; onsite measurements

Water type	Dissolved oxygen (%)	Salinity (PSU)	Tempera-ture (°C)	pH	Turbidity (NTU)	UVT (%)
Inlet water (control)	71 (±1.2)	34.3 (-)	22 (-)	7.9 (-)	4.8 (-)	87 (±0.3)
Inlet water (treated)	78 (±11.0)	33.9 (±0.2)	22.4 (±0.6)	7.9 (-)	5.5 (-)	87 (±1.0)
Control discharge	76 (±0.4)	34.4 (-)	19.5 (±0.1)	7.8 (-)	5.5 (-)	90 (±1.1)
Treated discharge	73 (±0.8)	34.1 (-)	21.3 (±0.1)	7.8 (-)	7.6 (±6.6)	89 (±4.3)

* Standard deviations in parentheses
PSU Practical salinity units
NTU Nephelometric turbidity unit
UVT UV transmittance

APPENDIX B

Detailed data for physical and chemical properties and biological efficacy analyses in shipboard testing of BIO-SEA

Physical-chemical parameters

Table B.1 Measurements of total suspended solids (TSS)

Test cycle	Water type	TSS (mg/L)				
		FR1	FR2	FR3	AVG	STD
#1	Inlet	161	153	120	**145**	22
	Treated water (T0)	n.d.	n.d.	n.d.	**-**	-
	Control discharge	n.d.	n.d.	n.d.	**-**	-
	Treated discharge	n.d.	n.d.	n.d.	**-**	-
#2	Inlet	61	63	106	**77**	25
	Treated water (T0)	44	41	47	**44**	3.2
	Control discharge	9.0	8.0	8.0	**8.3**	0.04
	Treated discharge	95	18	18	**43**	45
#3	Inlet	15	13	16	**14**	1.6
	Treated water (T0)	20	18	18	**19**	1.2
	Control discharge	17	13	14	**15**	2.3
	Treated discharge	17	17	11	**15**	3.1
#4	Inlet	16	17	17	**17**	0.4
	Treated water (T0)	17	17	15	**16**	1.4
	Control discharge	12	13	14	**13**	1.3
	Treated discharge	16	17	38*	**24**	12

FR Field replicate
AVG Average
STD Standard deviation
* An extreme high turbidity was measured during sampling of FR3
n.d. Not determined

Table B.2 Measurements of particulate organic carbon (POC)

Test cycle	Water type	POC (mg/L)				
		FR1	FR2	FR3	AVG	STD
#1	Inlet	1.9	1.6	2.2	**1.9**	0.34
	Control discharge	n.d.	n.d.	n.d.	**-**	-
	Treated discharge	n.d.	n.d.	n.d.	**-**	-
#2	Inlet	0.87	1.4	1.1	**1.1**	±0.26
	Control discharge	0.56	0.57	0.48	**0.53**	±0.05
	Treated discharge	0.90	0.81	0.84	**0.85**	±0.05
#3	Inlet	0.87	<0.1	<0.1	**0.46**	±0.58
	Control discharge	<0.1	<0.1	<0.1	**<0.1**	-
	Treated discharge	<0.1	<0.1	<0.1	**<0.1**	-
#4	Inlet	<0.1	<0.1	<0.1	**<0.1**	-
	Control discharge	<0.1	<0.1	0.11	**<0.1**	-
	Treated discharge	<0.1	<0.1	<0.1	**<0.1**	-

FR Field replicate
AVG Average
STD Standard deviation
n.d. Not determined

Table B.3 Measurements of dissolved organic carbon (DOC)

Test cycle	Water type	DOC (mg/L)				
		FR1	FR2	FR3	AVG	STD
#1	Inlet	2.4	2.4	2.4	**2.4**	0.03
	Control discharge	n.d.	n.d.	n.d.	**-**	-
	Treated discharge	n.d.	n.d.	n.d.	**-**	-
#2	Inlet	0.53	0.21	0.23	**0.32**	±0.18
	Control discharge	0.46	0.52	0.57	**0.52**	±0.06
	Treated discharge	0.25	0.30	0.32	**0.29**	±0.03
#3	Inlet	1.3	1.1	1.2	**1.2**	±0.10
	Control discharge	1.3	1.3	1.3	**1.3**	±0.01
	Treated discharge	1.2	1.1	1.3	**1.2**	±0.11
#4	Inlet	1.2	1.1	0.89	**1.1**	±0.17
	Control discharge	0.78	0.79	0.79	**0.79**	±0.01
	Treated discharge	0.93	0.78	0.83	**0.85**	±0.08

FR Field replicate
AVG Average
STD Standard deviation
* Sample lost
n.d. Not determined

Organism size class ≥50 µm

Table B.4 Enumeration of organisms ≥50 µm and sample volumes

Test cycle	Water type	Organisms ≥50 µm							
		FR1		FR2		FR3		AVG	STD
		vol. m³	org./m³	vol. m³	org./m³	vol. m³	org./m³	org./m³	
#1	Inlet	1.167	12,748	1.195	*	1.208	12,639	**12,694**	77
	Treated water (T0)	1.0	990**	n.d.	n.d.	n.d.	n.d.	**990****	-
	Control discharge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
	Treated discharge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-
#2	Inlet	1.100	30,039	1.100	23,591	1.044	24,360	**25,997**	3,522
	Treated water (T0)	1.094	0.91	1.100	1.8	1.090	1.8	**1.5**	0.53
	Control discharge	1.033	15,744	1.044	13,300	1.017	12,947	**13,997**	1,523
	Treated discharge	3.177	1.3	3.100	1.9	3.174	1.6	**1.6**	0.34
#3	Inlet	1.044	5,251	1.064	5,901	1.054	5,122	**5,425**	418
	Treated water (T0)	1.307	0.77	1.323	2.3	1.311	1.5	**1.5**	0.75
	Control discharge	1.086	5,731	1.089	6,633	1.054	7,581	**6,648**	925
	Treated discharge	3.202	3.4	3.190	1.9	3.209	0.93	**2.1**	1.3
#4	Inlet	1.320	177,455	1.320	180,352	1.318	167,334	**175,047**	6,835
	Treated water (T0)	1.556	4.5	1.554	10.4	1.501	7.5	**7.5**	2.9
	Control discharge	1.146	49,018	1.157	50,772	1.145	44,473	**48,088**	3,251
	Treated discharge	3.308	2.1	3.265	1.5	3.281	4.3	**2.6**	1.5

FR Field replicate
AVG Average
STD Standard deviation
n.d. Not determined
* Sample lost due to broken string on sampling net
** Only one replicate counted

Organism size class ≥10 and <50 µm

Table B.5 Enumeration of organisms ≥10 µm and <50 µm by microscopy using Lugol preservation (inlet) and CMFDA/FDA viable stain (control and treated discharge), respectively. * numbers in parenthesis represent viable organisms within the size 6-9 µm.

Test cycle	Water type	Organisms/mL				
		FR1	FR2	FR3	AVG	STD
#1	Inlet (Lugol)	195	163	116	**158**	40
	Control discharge (CMFDA/FDA)	n.d.	n.d.	n.d.	**-**	-
	Treated discharge (CMFDA/FDA)	n.d.	n.d.	n.d.	**-**	-
#2	Inlet (Lugol)	74 (+41)*	76 (+42)*	104 (+15)*	**85 (+33)***	±16.8 (±15.3)
	Control discharge (CMFDA/FDA)	9.3	11	10.3	**10.2**	±0.9
	Treated discharge (CMFDA/FDA)	0.6	0.4	1.2	**0.73**	±0.42
#3	Inlet (Lugol)	517	455	537	**503**	±42
	Control discharge (CMFDA/FDA)	258	226	234	**239**	±17
	Treated discharge (CMFDA/FDA)	0.83	0	0.17	**0.33**	±0.4
#4	Inlet (Lugol)	832	717	800	**783**	±59
	Control discharge (CMFDA/FDA)	210	206	212	**209**	±4.1
	Treated discharge (CMFDA/FDA)	2.5	0.83	1.8	**1.7**	±0.84

FR Field replicate
AVG Average
STD Standard deviation
n.d. Not determined

Table B.6 Determination of viable algae by the re-growth assay

Test cycle	Water type	Viable algae (organisms/mL)									
		FR1	FR2	FR3	FR4	FR5	FR6	FR7	FR8	FR9	AVG
#1	Inlet	>160	>160	>160	-	-	-	-	-	-	**>160**
	Control discharge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**-**
	Treated discharge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**-**
#2	Inlet	160	>160	>160	-	-	-	-	-	-	**>160**
	Control discharge	>160	>160	>140	-	-	-	-	-	-	**>153**
	Treated discharge	<0.18	<0.18	<0.18	<0.18	<0.18	<0.18	<0.18	<0.18	<0.18	**<0.18**
#3	Inlet	>160	>160	>160	-	-	-	-	-	-	**>160**
	Control discharge	>160	>160	>160	-	-	-	-	-	-	**>160**
	Treated discharge	2.3 (0.9-6.1)	0.20 (0.03-1.4)	0.45 (0.11-1.8)	0.20 (0.03-1.4)	0.18 (0.02-1.4)	<0.18	<0.18	<0.18	<0.18	**0.45**
#4	Inlet	>160	>160	>160	-	-	-	-	-	-	**>160**
	Control discharge	>160	>160	>160	-	-	-	-	-	-	**>160**
	Treated discharge	0.20 (0.02-1.4)	0.78 (0.25-2.4)	0.20 (0.03-1.4)	<0.18	<0.18	0.45 (0.11-1.8)	<0.18	0.20 (0.03-1.4)	<0.18	**0.28**

FR Field replicate
AVG Average
( ) 95% confidence interval
n.d. Not determined

Bacteria

Table B.8 Enumeration of *E. coli*

Test cycle	Water type	*E. coli* (CFU/100 mL)										
		FR1	FR2	FR3	FR4	FR5	FR6	FR7	FR8	FR9	AVG	STD
#1	Inlet	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Control discharge	n.d.	n.d.	n.d.	-	-	-	-	-	-	**-**	-
	Treated discharge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**-**	-
#2	Inlet	43	21	65	-	-	-	-	-	-	**43**	±14
	Control discharge	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Treated discharge	<10	<10	<10	<10	<10	<10	<10	<10	<10	**<10**	-
#3	Inlet	21	<10	<10	-	-	-	-	-	-	**14**	-
	Control discharge	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Treated discharge	<10	<10	<10	<10	<10	<10	<10	<10	<10	**<10**	-
#4	Inlet	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Control discharge	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Treated discharge	<10	<10	<10	10	<10	<10	<10	<10	<10	**<10**	-

CFU Colony-forming units
FR Field replicate
AVG Average
STD Standard deviation
n.d. Not determined

Table B.9 Enumeration of enterococci

Test cycle	Water type	Enterococci (CFU/100 mL)										
		FR1	FR2	FR3	FR4	FR5	FR6	FR7	FR8	FR9	AVG	STD
#1	Inlet	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Control discharge	n.d.	n.d.	n.d.	-	-	-	-	-	-	**-**	-
	Treated discharge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**-**	-
#2	Inlet	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Control discharge	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Treated discharge	<10	<10	<10	<10	<10	<10	<10	<10	<10	**<10**	-
#3	Inlet	<10	<10	10	-	-	-	-	-	-	**<10**	-
	Control discharge	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Treated discharge	<10	<10	<10	<10	<10	<10	<10	<10	<10	**<10**	-
#4	Inlet	10	<10	<10	-	-	-	-	-	-	**<10**	-
	Control discharge	<10	<10	<10	-	-	-	-	-	-	**<10**	-
	Treated discharge	10	<10	<10	<10	<10	<10	<10	<10	<10	**<10**	-

CFU Colony-forming units
FR Field replicate
AVG Average
STD Standard deviation
n.d. Not determined

Table B-10 Enumeration of *Vibrio cholerae*

Test cycle	Water type	*Vibrio cholerae* (CFU/100 mL)										
		FR1	FR2	FR3	FR4	FR5	FR6	FR7	FR8	FR9	AVG	STD
#1	Inlet	<1	<1	<1	-	-	-	-	-	-	-	-
	Control discharge	n.d.	n.d	n.d	-	-	-	-	-	-	-	-
	Treated discharge	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	-	-
#2	Inlet	<1	<1	<1	-	-	-	-	-	-	-	-
	Control discharge	<1	<1	<1	-	-	-	-	-	-	-	-
	Treated discharge	<1	<1	<1	<1	<1	<1	<1	<1	<1	-	-
#3	Inlet	<1	<1	<1	-	-	-	-	-	-	-	-
	Control discharge	<1	<1	<1	-	-	-	-	-	-	-	-
	Treated discharge	<1	<1	<1	<1	<1	<1	<1	<1	<1	-	-
#4	Inlet	<1	<1	<1	-	-	-	-	-	-	-	-
	Control discharge	<1	<1	<1	-	-	-	-	-	-	-	-
	Treated discharge	<1	<1	<1	<1	<1	<1	<1	<1	<1	-	-

CFU Colony-forming units
FR Field replicate
AVG Average
STD Standard deviation
n.d. Not determined

APPENDIX C

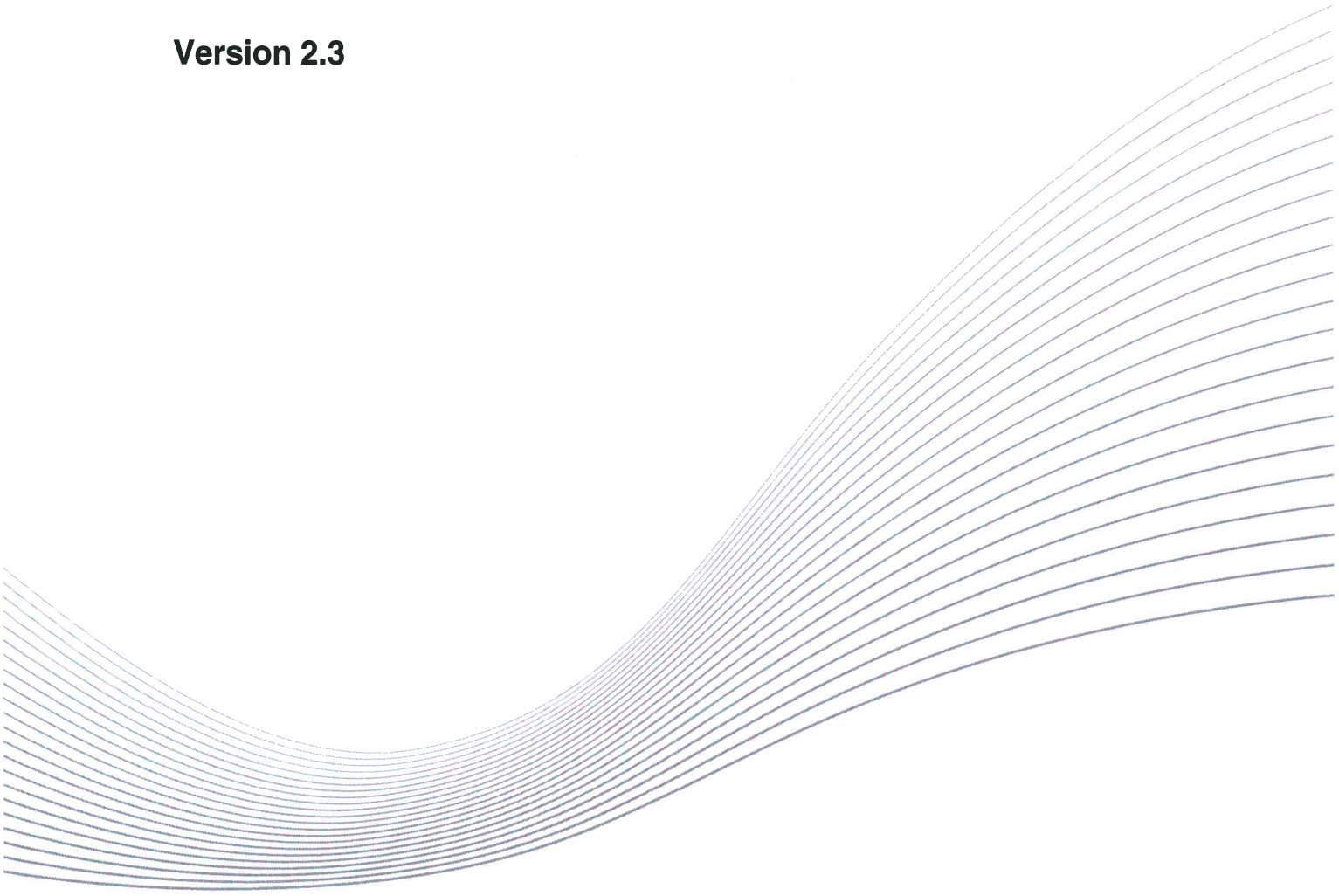
QMP and QAPP with Amendment No. 1

Quality Management Plan

Performance Evaluation of Ballast Water Management Systems

DHI Denmark

Version 2.3



WATER • ENVIRONMENT • HEALTH

September 2011

Quality Management Plan

Performance Evaluation of Ballast Water Management Systems

DHI Denmark
Version 2.3

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Project	Project No.
Quality Management Plan Performance Evaluation of Ballast Water Management Systems DHI Denmark Version 2.3	
Author Gitte I. Petersen	Date 2011.09.07
	Approved by Torben Madsen

Revision	Description	By	Checked	Approved	Date
2.3	QMP	GIP	TMA	TMA	7/9-11

Key words	Classification
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Distribution	No. of copies
Client Certification body DHI: /EAT	

CONTENTS

APPENDICES

1 TERMS AND ABBREVIATIONS

Terms/Abbreviations	Definitions and comments
Active substance	A substance which has a general or specific action on aquatic organisms or bacteria (pathogens)
Ballast Water Management System (BWMS)	A system which removes, renders harmless or avoids uptake or discharge of aquatic organisms and bacteria (pathogens) with ballast water and sediments by mechanical, physical, chemical or biological means acting individually or in combination
Certification Body	Body to certify facilities to conduct performance evaluation of BWMS according to the IMO Convention
Client	The party requesting a performance evaluation of a technology.
Convention	The IMO convention on ballast water
International Maritime Organization (IMO)	United Nations specialized agency with responsibility for the safety and security of shipping and the prevention of marine pollution by ships Comment: IMO has adopted the International Convention for the Control and Management of Ship's Ballast Water and Sediments
Quality Assurance Project Plan (QAPP)	Project-specific technical document describing the BWMS to be tested, the test facility and other conditions affecting the actual design and implementation of the required experiments
Quality Management Plan (QMP)	Generic document describing the quality control management structure and policies of the testing body (including subcontractors and outside laboratories)
Services	The performance evaluation of maritime technologies by laboratory, land-based or shipboard tests or a combination hereof
Standard Operation Procedure (SOP)	Generic document providing rules, guidelines or characteristics for tests or analyses Comment: In-house methods may be used in the absence of a recognized standard, if they are commonly accepted for testing of BWMS or scientifically documented

2 INTRODUCTION

The International Maritime Organization (IMO) has adopted the International Convention for the Control and Management of Ship's Ballast Water and Sediments /1/ to reduce the risk of spreading of harmful aquatic organisms and pathogens released with ballast water.

The Convention requires that all ships comply with specified water quality requirements (D2) before ballast water is released into the environment.

The performance evaluation of ballast water management systems (BWMS) aims at documenting compliance with the requirements stated in international guidelines, e.g.:

- Guideline for approval of ballast water management systems - G8 /2/

- Procedure for approval of ballast water management systems that make use of active substances - G9 /3/.

DHI provides services in relation to performance evaluation of maritime technologies and particularly BWMS within the DHI Ballast Water Centre which includes test facilities and laboratories in Denmark and Singapore.

The DHI Ballast Water Centre is organized with a Ballast Water Facility Board including two members from the management in DHI Denmark and two members from the management in DHI Singapore. The object of the Board is to coordinate the development and marketing of services related to the performance evaluation of BWMS within the DHI Group.

The services addressed with the present Quality Management Plan (QMP) include:

- Laboratory tests conducted at the DHI environmental laboratory in Hørsholm, Denmark, aiming at proof-of-concept or technology optimisation
- Pilot-tests conducted at the DHI Maritime Technology Evaluation Facility (hereafter referred to as the "test facility") in Hundested, Denmark, aiming at technology optimisation
- Land-based tests conducted at the test facility according to international guidelines
- Shipboard tests conducted by DHI Denmark according to international guidelines at vessels, on which the technology is installed

The above activities are collectively referred to as the "services" whereas individual activities are referred to as "projects".

The aim of the services is to provide independent, third party documentation for the performance of maritime technologies. High quality of the services is ensured through extensive quality management and use of skilled staff.

3 ORGANISATION

3.1 Head of department (Torben Madsen)

The head of department, business strategy, for DHI's Department of Environment and Toxicology, has the overall responsibility for the services and the test facility. This includes the following tasks:

- Co-ordination of joint business development between DHI Denmark and DHI Singapore via participation in the Ballast Water Facility Board
- Negotiation of agreements (i.e. service contracts) with clients
- Responsibility for overall co-ordination, planning and costs as required to ensure that the appropriate human resources, facilities and equipment are available for the services
- Appointment of the business area manager, the project manager and task leaders for cross-cutting functions (e.g. production of test water and test facility technical operations)
- Maintenance of the QMP with updated versions as appropriate

- Approval of the Quality Assurance Project Plan (QAPP) and Standard Operation Procedures (SOPs)
- Quality control and approval of test reports (provided that the head of department has not contributed to the technical solution of the project)
- Documentation in relation to

    - Staff training and experience
    - Facilities and their maintenance
    - Records of complaints

3.2 Business area manager (Gitte I. Petersen)

The business area manager is responsible for the scientific and technical quality of the services in co-ordination with the head of department. This includes the following managerial tasks:

- Business development and marketing
- Maintenance of generic standards that can serve as formats for drafting the QAPPs and approval of the methods applied in land-based and shipboard tests
- Dialogue with task leaders for cross-cutting functions, e.g. production of test water and test facility technical operations
- Contributions to data interpretation and reporting of land-based and shipboard tests in collaboration with the project manager
- Participation in discussions with the Certification Body on important matters, particularly draft and final reports, together with the project manager
- Co-ordination of the services with the aim to ensure feasibility of parallel projects conducted at the test facility, including decisions related to the functioning of the test facility (e.g. piping and pumps)
- Maintenance of the test facility, connection piping between the test facility and the client's technology, and dialogue with academic and technical staff in order to fulfil DHIs responsibility for operating the test facility during testing
- Quality control of test reports (provided that the business area manager has not contributed to the technical solution of the project)

3.3 Project manager

The project manager is responsible for the management and efficient performance of the project in accordance with the contract between the client and DHI, the QMP and the QAPP.

The project manager's tasks include:

- Organisation and management of the project
- Periodic meetings and other communication with the client to ensure that all necessary information is available in due time
- Preparation of the draft and final QAPP with detailed description of the project, including time schedule and quality assurance of deliverables

- Facilitation of the process for comments and responses to the draft QAPP in dialogue with the client and the Certification Body
- Preparation of amendments and deviations to the QAPP, if any
- Communication of the project time schedule to the Certification Body to enable external audit
- Communication of the QAPP and project time schedule to the internal auditor identified in the QAPP to enable internal audit
- Participation in discussions with the Certification Body on important matters, particularly draft and final reports, together with the business area manager
- Co-ordination and dialogue with the business area manager in relation to safe conditions of work, logistics and technical operations at the test facility
- Co-ordination and dialogue with the laboratory manager in relation to the practical organisation of work involving laboratory technicians; the project manager shall in due time inform the laboratory manager on the types of tests and the required capacity to enable laboratory capacity planning
- Agreements with subcontractors as appropriate for meeting the project deliverables (e.g. chemical analytical laboratory)
- Approval of initiation of the test cycles and interruption of test cycles, e.g. in case of irregularity
- Preparation of reports

3.4 Head of projects

The academic staff (with exception of the business area manager, project manager, task leaders for cross-cutting functions and test co-ordinators) and the secretaries are appointed by the head of projects via dialogue with the business area manager or the project manager as appropriate.

3.5 Laboratory manager

The laboratory manager appoints laboratory technicians for a specific project and allocates tasks to them as part of the laboratory capacity planning. Furthermore, the laboratory manager appoints one or more test co-ordinators among the laboratory technicians or the academic staff for on-site co-ordination of land-based test cycles.

3.5.1.1 Academic staff, laboratory technicians and secretaries

The tasks of the academic staff, the laboratory technicians and the secretaries include:

- Contributions to the QMP, QAPP and SOPs
- Test co-ordinator function, i.e. co-ordination and keeping timely records of the activities at the test facility during land-based tests
- Sampling at the test facility
- Monitoring of test water quality
- Maintenance of materials and equipment
- Analysis and data processing
- Contributions to test reports

- Archiving of documents and raw data

4 PERFORMANCE OF PROJECT

4.1 Agreement

An agreement between the client and DHI is negotiated and signed according to the DHI manual for project management.

4.2 Quality Assurance Project Plan (QAPP)

The QAPP is a project specific document describing the technology to be tested, the test facility, and other conditions affecting the actual design and implementation of the study. The QAPP is only required for performance evaluation of BWMS in land-based or shipboard tests conducted according to international guidelines.

The QAPP is

- Prepared by the project manager
- Signed by the project manager, the head of department and the internal auditor from the DHI Quality Assurance Unit
- Forwarded to the Certification Body for review and comments
- Forwarded to the client for review, acceptance and signature.

The QAPP typically includes the following titles:

1. Objective
2. Client (including client's monitor, if any)
3. Administration
4. DHI Ballast Water Centre
5. Subcontractors
6. Project management
7. System description
8. Safety handling of active substances
9. Test design (including, for **land-based test**, test cycles, test water, sampling and analyses, and, for **shipboard test**, trial period and locations, sampling and analyses)
10. Validity criteria
11. Pass criteria
12. Time schedule
13. Quality assurance
14. Report
15. Archiving
16. Amendments and deviations, if any
17. References

The QAPP refers to a number of SOPs (see Appendix A).

Amendments and deviations to the QAPP are approved and signed by the project manager. Amendments describe planned changes whereas deviations describe unplanned changes to the QAPP.

The QAPP is subject to internal audit in accordance with the procedures for internal audit of the DHI Quality Management System.

4.3 Services

The project will be conducted as described in the QAPP and subsequent amendments and deviations or, alternatively, as described in the agreement between the client and DHI for projects, for which no QAPP is prepared.

4.3.1 Laboratory tests

Laboratory tests can be initiated when the BWMS technology is ready for testing and DHI's deliverables are defined. Initiation of testing is decided by the project manager in agreement with the client.

4.3.2 Pilot tests

Pilot tests can be initiated when the BWMS technology is installed and ready for operation. Initiation of testing is decided in consensus by and between the business area manager and the project manager in agreement with the client.

4.3.3 Land-based tests

Land-based tests can be initiated when the BWMS technology is installed and ready for operation. Initiation of testing is decided in consensus by and between the business area manager and the project manager in agreement with the client.

The project manager decides when a test cycle in the land-based test is completed and valid, when appropriate by reference to the G8 guidelines /2/, G9 guidelines /3/ or other standards (e.g. US requirements). If required, the project manager can decide to interrupt a test cycle due to technical malfunctioning of the test facility or the BWMS, insufficient state of biological or physical parameters or for other reasons related to the quality of the test water.

4.3.4 Shipboard tests

Shipboard testing can be initiated when the BWMS technology is installed on the vessel and ready for operation. Initiation of testing is decided by the project manager in agreement with the client.

The project manager decides when a test cycle in the shipboard test is completed and valid by reference to the criteria in G8 /2/ or, if appropriate, to criteria in other standards (e.g. US requirements). If required, the project manager can decide to interrupt a test cycle due to technical malfunctioning of the BWMS, insufficient state of biological or physical parameters or for other reasons related to the water quality.

4.4 Reports

Reports are prepared with the details, format and language described in the agreement between the client and DHI.

4.4.1 Performance evaluation of BWMS under the IMO convention

For land-based or shipboard tests of BWMS conducted as part of the IMO approval process, the report is typically structured by use of the appropriate headings in the QAPP and shall include a summary of any amendments and deviations to the QAPP.

The report shall include all relevant technical and analytical data and will contain at least the following items:

- Name and address of the client (and monitor, if any)
- Name and address of the testing facility and the dates, on which the test was initiated and completed
- Objectives and procedures stated in the approved QAPP including any changes made to the QAPP
- Results obtained, presented in summarizing tables and as raw data
- Any unforeseen circumstances which may have affected the quality or integrity of the land-based/shipboard testing
- Storage locations of all raw data, the signed QAPP and report
- Descriptions of operations, calculations and transformations performed on the presented data
- Quality assurance statement

The report shall be signed by the project manager, the internal auditor from the DHI Quality Assurance Unit and the head of department.

The final report will be prepared in English and forwarded to the client.

5 QUALITY MANAGEMENT PROCESSES

5.1 DHI Quality Assurance

The services are conducted in accordance with the principles of ISO 9001 by using the DHI Quality Manual and the procedures in this QMP. The Quality Management System of DHI is found compliant with ISO 9001 as part of the ISO 17025 accreditation of the DHI environmental laboratory.

The DHI quality manager is responsible for assigning a trained internal auditor from DHI's Quality Assurance Unit to each project in accordance with the procedures for internal audit of the DHI Quality Management System.

The internal auditor is identified in the QAPP. The internal auditor shall receive the QAPP from the project manager in order to plan and execute internal audit of the project.

5.2 Document and record control

The DHI Quality Manual includes a procedure describing the process of drafting, revising and approving documentation. Standard operation procedures are controlled as described in SOP 30/944.

SOPs 30/921 and 30/937 describe how records of the test are stored, transferred, maintained and controlled in order to ensure data integrity for a period defined in the QAPP, but not shorter than 5 years from completion of the verification.

5.3 Internal audits

Procedure 3 in the DHI Quality System Manual on audit and evaluation and SOP 30/943 describe the process of periodic internal auditing of projects and activities including audit responsibilities and planning, auditor training and competences and audit reporting.

Procedure 4 in DHI Quality System Manual on non-conformities and corrective actions describes how deviations identified during operation and auditing are corrected (corrective actions) and how future occurrence of the same deviations is prevented by improving the quality manual including the process descriptions and working methods (preventive actions).

5.4 Complaint management

Procedure 5 in the DHI Project Management Manual on Complaints describes how complaints are recorded, resolved and reported. If not resolved, complaints are referred to the Certification Body for resolving.

5.5 Subcontractor management

Procedure 4 in the DHI Project Management Manual on subcontractors describes how it is ensured that subcontractors follow quality requirements.

In addition, analytical laboratories providing analyses of any kind should:

- Maintain an ISO 17025 accreditation with the quality management system required herein.
- Apply accredited analytical methods when available.
- Apply other methods according to either international standard methods or in-house methods that are in all cases validated as required for accredited methods.

SOP 30/700 furthermore describes how it is ensured that purchased items such as chemicals and glassware are controlled, accepted and calibrated.

5.6 Staff competence management

Procedure 3 on appraisal interview, post qualifying education and experience in the DHI Employee Conditions Handbook describes how it is ensured that the projects are conducted by staff with adequate competences and knowledge. This is done by maintaining a list of functions in the test process with competence requirements and responsibilities. The list is supported by reference to staff files in the DHI CV database.

5.7 Facility management

SOP 30/945 describes how it is ensured that facilities and equipment are available and fit for the purposes.

5.8 Management review

Procedure 3 of the Quality System Manual on audit and evaluation describes how the DHI management is ensuring that the test centre is working according to this quality manual through mechanisms such as e.g. an annual management review process.

The Quality Manager is responsible for maintenance and development of the quality system and for the internal auditing of all aspects of the system – with daily reference to the Director, Group R&D and Quality Management. The DHI Quality Manual contains rules for reviews of the quality system.

6 REFERENCES

/1/ IMO (2005): International Convention for the Control and Management of Ships Ballast Waterand Sediments. London. International Maritime Organization.

/2/ MEPC. Guidelines for approval of ballast water management systems (G8). resolution MEPC.174(58). Adopted 10th October 2008.

/3/ MEPC. Procedure for approval of ballast water management systems that make use of active substances (G9). MEPC.126(53) Adopted 22nd July 2005.

A P P E N D I X A

BMWS testing-specific Standard Operating Procedures (SOPs)

SUBJECT/SUBSUBJECT	NO.
ANALYTICAL METHOD ZOOPLANKTON ANALYSIS	30/1700:04
ANALYTICAL METHOD MICROSCOPIC ENUMERATION AND IDENTIFICATION OF MICROALGAE (LUGOL AND CMFDA/FDA)	30/1701:02
ANALYTICAL METHOD DETERMINING PRIMARY PRODUCTION OF MICROALGAE	30/1702:03
ANALYTICAL METHOD DETERMINING DIVERSITY OF MICROALGAL COMMUNITIES BY HPLC ANALYSIS OF PIGMENTS	30/1703:03
ANALYTICAL METHOD DETERMINATION OF VIABLE ALGAE BY MPN	30/1704:02
MICROBIOLOGICAL TESTS DETERMINATION OF TOTAL NUMBER OF BACTERIA BY EPIFLUORESCENCE MICROSCOPY	30/1705:03
MICROBIOLOGICAL TESTS DETERMINATION OF HETEROTROPHIC PLATE COUNT	30/1706:03
MICROBIOLOGICAL TESTS DETERMINATION OF *VIBRIO CHOLERAE* IN WATER	30/1707:02
MICROBIOLOGICAL TESTS DETERMINATION OF TOTAL COLIFORM, *E.COLI* AND ENTEROCOCCI Colilert*-18 AND Enterolert-E	30/1708:02
MEASUREMENT METHOD OZONE MEASUREMENT IN WATER	30/1730:02
MEASUREMENT METHOD OZONE MEASUREMENT IN AIR	30/1731:02
MEASUREMENT METHOD TRO MEASUREMENT IN WATER	30/1732:02
HARVESTING, CULTURING AND ADDITION OF ORGANISMS	30/1734:03
COLLECTION OF SEAWATER	30/1735:02
COLLECTION OF FRESH WATER	30/1736:02
CHEMICAL CRITERIA FOR TEST WATER ADDITION OF DOC, POC AND MM	30/1737:02
SAMPLING BIOLOGICAL AND WATER QUALITY PARAMETERS	30/1738:02
SAMPLING WET TEST	30/1739:02
SAMPLING DBP ANALYSIS	30/1740:02
STATISTICS STATISTICAL SURVEILLANCE OF BIOLOGICAL DATA OBTAINED AT TESTS OF BWMSs	30/1760:01
LABELLING SAMPLES COLLECTED AT TEST SITE	30/1761:01
OPERATION OF THE DHI MTEF	30/1762:02
CLEANING RETENTION TANKS, PIPINGS AND OTHER EQUIPMENT AT TEST SITE	30/1763:02
MEASUREMENT METHOD ON-LINE MONITORING OF PRESSURE, TEMPERATURE AND FLOW RATES AT TEST SITE	30/1764:01
MEASUREMENT METHOD FLUORESCENCE	30/1765:02

SUBJECT/SUBSUBJECT	NO.
MEASUREMENT METHOD TURBIDITY	30/1766:03
HEALTH AND SAFETY ENSURING WORKER HEALTH AND SAFETY AT TEST SITE	30/1767:02
MEASUREMENT METHOD DETERMINATION OF TSS	30/1768:02
MEASUREMENT METHOD DETERMINATION OF DOC AND POC	30/1769:02

APPENDIX B

Overview of lists

Overview of lists

The lists mentioned below are kept together with the rest of quality documentation.

Certification Body

DHI holds a statement describing the Certification Body that has certified the DHI Maritime Technology Evaluation Facility.

List of sub-contractors

DHI keeps a list of sub-contractors used during the test. The list contains information on name of company, address, contact person, e-mail, telephone number and deliveries.

List of staff approved for functions at the test facility

DHI keeps a list of persons working at the test facility. The list contains information on the person's activities, responsibility and documentation for training. The person's competence is documented in an available CV.

List of Standard Operation Procedures

DHI keeps a list of SOPs, including those used in relation to projects conducted at the test facility.

APPENDIX C

Template for amendments to QAPP

AMENDMENT

QAPP DOCUMENT TITLE AND DATE:

AMENDMENT NUMBER:

DATE OF AMENDMENT:

AMENDMENT CONTENTS:

REASON FOR AMENDMENT:

IMPACT OF AMMENDMENT:

PREVENTATIVE ACTION:

If relevant, action to prevent that the same cause of amendment will reoccur in the future.

ORIGINATED BY:

SIGNED BY:

_____

Project manager

_____

DATE

Copy to be sent to the client, the Certification Body and the DHI Quality Assurance Unit.

APPENDIX D

Template for amendments to QAPP

DEVIATION

QAPP DOCUMENT TITLE AND DATE:

DEVIATION NUMBER:

DATE OF DEVIATION:

DESCRIPTION OF DEVIATION:

REASON FOR DEVIATION:

IMPACT OF DEVIATION:

CORRECTIVE ACTION:

If required, actions to be taken to prevent consequences of deviation

ORIGINATED BY:

SIGNED BY:

_____
Project manager

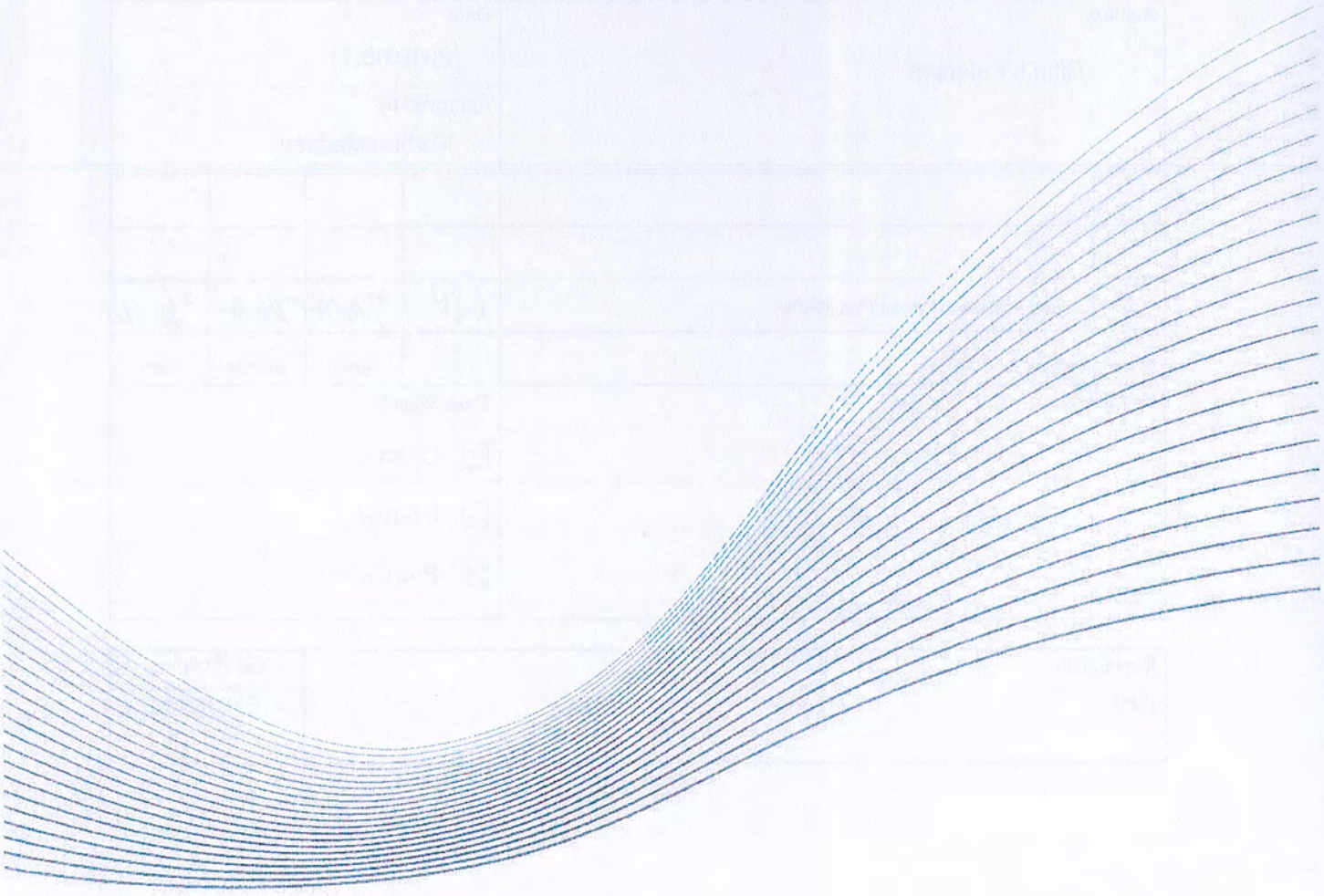
_____
DATE

Copy to be sent to the client, the Certification Body and the DHI Quality Assurance Unit.

QAPP to:
BIO-UV

Quality Assurance Project Plan

Shipboard Test of BIO-UV's BIO-SEA Ballast Water Management System



WATER • ENVIRONMENT • HEALTH

June 2012

Quality Assurance Project Plan

Shipboard Test of BIO-UV's BIO-SEA Ballast Water Management System

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Client	Client's representative
BIO-UV	Charlène Ceresola

Project	Project No
Shipboard Test of BIO-UV's BIO-SEA Ballast Water Management System	11812218
Authors: Gitte I. Petersen	Date: 2012.06.11
	Approved by: Torben Madsen

Revision	Description	By	Checked	Approved	Date
01	Quality Assurance Project Plan (QAPP)	GIP	TMB	TMB	11/6-12

Key words	Classification
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Distribution	No of copies
DHI	Electronic

CONTENTS

APPENDICES

1 OBJECTIVE

For an application for final approval, the IMO Convention requires an approval of Ballast Water Management Systems (BWMS) according to the principles laid down in Resolution MEPC.174(58) (G8) /1/ to assure that BWMS approved by administrations are capable of meeting the standard regulation D-2 (MEPC G8) in land-based and shipboard evaluations and do not cause unacceptable harm to the vessel, crew, environment or public health.

The objective is to conduct a shipboard test of BIO-UV's BIO-SEA BWMS (hereafter BIO-SEA) according to Resolution MECP.174(58), Guidelines for approval of ballast water management systems (G8) (hereafter designated as the 'guidelines').

2 CLIENT

Bio-UV
245 rue de la ZAC de la Petite Camargue
CS90022 – 34403 LUNEL Cedex
France

Contact person: Charlène Ceresola

3 CLASSIFICATION SOCIETY

Bureau Veritas
Division Marine – Direction Technique
61 – 67 Boulevard du Château
92200 Neuilly sur Seine
France

Contact person: Christian Robeson

4 DHI BALLAST WATER CENTRE

DHI
Agern Allé 5
DK-2970 Hørsholm
Denmark

Contact Person: Gitte I. Petersen

5 SUBCONTRACTORS

The shipboard test will be conducted by DHI, and, with the possible exception of verification of *Vibrio cholerae* (according to SOP 30/1707), subcontractors will not be involved.

6 PROJECT MANAGEMENT

The project manager for the study is Gitte I. Petersen.

7 DESCRIPTION OF BALLAST WATER MANAGEMENT SYSTEM

The description of BIO-SEA, provided in Appendix A, is identical to the description received from the client.

8 SAFETY HANDLING OF ACTIVE SUBSTANCES

No active substances are applied by the BWMS.

9 EXPERIMENTAL DESIGN

9.1 Trial period and locations

The shipboard test will include three test cycles conducted during at least two separate campaigns on board the CMA CGM container vessel MOZART. The campaigns will be conducted within a trial period with a time span of not less than six months.

The campaigns are planned to be conducted in ports in China, South Korea and/or Malaysia.

The first campaign including one test cycle is scheduled to be conducted in June 2012 between Pusan (Korea) and Ningbo (China).

The second campaign including two test cycles is scheduled to be conducted in January 2013 or later. Details on dates and locations for ballasting and de-ballasting activities will be provided as amendments to the quality assurance project plan when this information is available.

9.2 Test cycles

The BWMS will be operated by BIO-UV during the three test cycles. Each test cycle consists of sampling and analyses of:

- **Inlet water** (the physico-chemical and biological parameters in the inlet water will be considered as sufficiently stable during the ballasting; unless the local conditions

indicate that the parameters in the inlet water vary with time, only one set of samples and analyses will be used to represent the control tank and the treated tank)

- **Control discharge water** (stored without treatment from the time of ballasting to discharge)
- **Treated discharge water** (treated and stored from the time of ballasting to discharge).

10 SAMPLING AND ANALYSIS PLAN

10.1 Sample overview

	Samples	Sample volumes per replicate
Inlet water	3 replicates	Organisms ≥50 µm: >1 m^3 *
		Organisms 10-50 µm: >1 L **
		Bacteria: >0.5 L **
		DOC + POC: approx. 0.5 L **
		TSS: approx. 2 L **
Control discharge water	3 replicates	Organisms ≥50 µm: >1 m^3 *
		Organisms 10-50 µm: >1 L **
		Bacteria: >0.5 L **
		DOC + POC: approx. 0.5 L **
		TSS: approx. 2 L **
Treated discharge water	3 replicates	Organisms ≥50 µm: >3 m^3 *
	3 x 3 replicates	Organisms 10-50 µm: >1 L **
	3 x 3 replicates	Bacteria: >0.5 L **
	3 replicates	DOC + POC: approx. 0.5 L **
	3 replicates	TSS: approx. 2 L **

* Collected by continuous flow during the entire period of intake or discharge; this continuous sampling of 3 replicates, each with a volume of at >3 m^3, provides the same statistical basis for counting as the sampling 3 x 3 replicates of >1 m^3 which is recommended in the guidelines

** Grab samples collected over the period of intake or discharge (e.g. start, middle and end)

In addition to the above mentioned parameters the turbidity of the inlet water will be measured by use of a portable turbidimeter brought by DHI. Furthermore the UV-transmittance (UV-T) will be measured by use of a spectrophotometer REALTECH UV254 P200 brought by BIO-UV. DHI will be responsible for the UV-T readings.

10.2 Samples for enumeration of organisms ≥50 µm

Three replicates will be collected by parallel continuous sampling during the entire periods of intake and discharge. The samples will be gently filtered through a net with a mesh size of 35 µm and a reservoir (cod-end) at the bottom of the net for collecting the zooplankton. Each replicate will be concentrated in 1-L glass bottles. The total volume of the filtered sample will be determined by a flow meter.

10.3 Samples for enumeration of organisms ≥10 µm and <50 µm

Grab samples (3 replicates for the inlet water, 3 replicates for the control discharge water, and 3 x 3 replicates for the treated discharge water) with a volume of at least 1 L will be collected in appropriate containers.

10.4 Samples for enumeration of bacteria

Grab samples (3 replicates for the inlet water, 3 replicates for the control discharge water, and 3 x 3 replicates for the treated discharge water) with a volume of at least 0.5 L will be collected in appropriate sterile containers.

11 ANALYSES

11.1 Analysis overview

Samples and replicates	Temperature ; Salinity; Turbidity and UV-T	≥ 50 µm	10-50 µm, Lugol's	10-50 µm, MPN	10-50 µm, micro-zooplankton	10-50 µm, Primary production	Bacteria	DOC + POC	TSS
Inlet water									
Rep 1 (start)	1	Three replicates	1	1		1	1	1	1
Rep 2 (mid)	2		2	2		2	2	2	2
Rep 3 (end)	3		3	3		3	3	3	3
Control discharge water									
Rep 1 (start)	1	Three replicates	1	1	1	1	1	1	1
Rep 2 (mid)	2		2	2	2	2	2	2	2
Rep 3 (end)	3		3	3	3	3	3	3	3
Treated discharge water									
Rep 1-3 (start)	1	Three replicates	1	1-3	1	1	1-3	1	1
Rep 4-6 (mid)	4		4	4-6	4	4	4-6	4	4
Rep 7-9 (end)	7		7	7-9	7	7	7-9	7	7

The samples for all analyses will be kept cool from the time of collection, and the samples will be processed for analyses within shortest possible time period.

11.2 Physical parameters

Temperature, salinity, turbidity and UV-T will be measured by use of portable multi parameter instruments equipped with electrodes. Measurements will be conducted at regular intervals throughout the inlet and discharge operations.

11.3 Organism size class ≥50 µm

Compliance with the pass criterion in Section 12 will be verified by using the direct count of organisms ≥50 µm.

The concentrations of viable organisms ≥50 µm in the samples will be determined by using a stereo microscope and a counting chamber according to SOP 30/1700. Viable organisms will be determined on the basis of mobility and morphology. The viable organisms will be characterized according to broad taxonomic groups such as rotifers, crustaceans, molluscs, worms, etc. The analyses will be completed on location.

11.4 Organism size class ≥10 µm and <50 µm

Compliance with the pass criterion in Section 12 will be verified by using the total of viable algae determined by the most probable number (MPN) assay and viable micro-zooplankton ≥10 and <50 µm.

11.4.1 Work on location

Samples preserved with Lugol's solution. Inlet and discharge water samples will be preserved with Lugol's solution to enable determination of the concentrations of organisms in the size class ≥10 and <50 µm. The container with inlet or discharge water sample will be shaken gently (upside down 5 times); a subsample (approx. 100 mL) per replicate will be transferred immediately to brown 100-mL glass bottles and Lugol's solution will be added to achieve a final concentration of 2% according to SOP 30/1701.

Most probable number (MPN) assay. The concentrations of viable algae in the inlet and discharge water samples will be analyzed by use of the MPN assay. The container with the total sample (> 2 L) will be shaken gently (upside down 5 times). One subsample (approx. 10 mL) of undiluted water per replicate will kept in darkness as 'back-up samples'. For the MPN assay, dilution series of the inlet water, control discharge water and treated discharge water will be prepared by adding 1 mL-aliquots of sample to test tubes with 5 mL of liquid medium as described in SOP 30/1704. Twenty (20) control test tubes containing only 5 mL of medium will be prepared (10 test tubes connected to inlet water and 10 test tubes connected to discharge water samples). The test tubes will be kept in the dark at a temperature between 10 and 30°C until arrival at the DHI laboratory.

Micro-zooplankton. To verify the presence of viable micro-zooplankton in the treated discharge water samples a volume of 5 mL is divided into subsamples with a volume of approx. 1 mL depending of the turbidity. The number of viable micro-zooplankton will be determined on the basis of mobility by using a stereo microscope and a counting chamber according to SOP 30/1701. Viable organisms will be determined on the basis of mobility and morphology. The analyses will be completed on location.

Primary production. For measuring the primary production of algae in inlet and discharge water samples, two representative subsamples of each replicate will be transferred to 60-mL bottles and incubated according to SOP 30/1702 for approx. 2 hours under light from a light-panel. The incubation will take place in a container at in situ temperature, and the bottles will be gently rotated every 15 min to ensure mixing of the algae. After incubation, the samples will be filtered onto GF/D filters and the filters will be transferred to glass vials as described in SOP 30/1702.

11.4.2 Work in laboratory

Samples preserved with Lugol's solution. These samples will be analysed as follows:

- Inlet water. Assuming that practically all of the organisms in the natural water are living, fulfilment of the validity criterion for the concentration of organisms ≥10 and <50 µm in the inlet water (Section 11) will be confirmed by inverted microscopy counting according to SOP 30/1701. The analyses comprise detailed examination of the algal chloroplasts to confirm that the phytoplankton were alive and classification of the algae according to groups, taxa or species.

- Control discharge water. Inverted microscopy counting will in combination with the MPN count (and primary production as a supporting parameter) be applied to confirm that the validity criterion for the concentration of organisms ≥10 and <50 µm in the control discharge water is fulfilled (Section 11).

- Treated discharge water. Inverted microscopy counting will be applied to quantify the predominant groups, taxa and species ≥10 and <50 µm in the treated discharge water with the purpose to add to the documentation of the MPN assay (see below).

Most probable number (MPN) assay. Upon arrival to the laboratory, the fluorescence of the test tubes will be determined before incubation (t_0). The test tubes will be incubated for 14 days at room temperature as described in SOP 30/1704. The concentrations of viable algae in the inlet water, control discharge water and treated discharge water will be determined by measuring the fluorescence in the test tubes according to SOP 30/1704.

The MPN assay will be documented by the growth of the naturally occurring algae under the conditions in the MPN assay. Identification of groups, taxa or species in the local water capable of growing under the conditions in the MPN assay will be performed with undiluted inlet water and after serial dilution. In addition, the algal groups, taxa or species in the inlet water will be thoroughly analyzed and compared with the list of algae capable of growing under the conditions in the MPN assay, which has been obtained during land-based and shipboard test cycles in Hundested, Denmark (some of the groups, taxa or species may be the same across geographic regions). DHI is confident that the MPN assay is conducted with an appropriate medium and under conditions that support the growth of a versatile range of algal species. However, the limited number of test cycles (1 or 2) conducted during a shipboard test voyage implies that the list of algae identified in the inlet water and in the MPN assay will be less comprehensive compared with the list obtained from the large number of test cycles in Hundested.

The algal groups, taxa and species in the Lugol's solution preserved treated discharge water samples will be compared with the identified algae capable of growing under the conditions in the MPN assay. This comparison will enable the confirmation or rejection of whether the predominant groups, taxa or species in the treated discharge water are able to grow under the conditions in the MPN assay (confirmation will mean that the results obtained in the MPN assay may be regarded as a valid quantification of the viable algae in the treated discharge water).

Primary production. Primary production will be determined by measuring the amounts of ^{14}C fixed by photosynthesis by liquid scintillation counting according to SOP 30/1702.

11.5 Bacteria

11.5.1 Work on location

One sample per replicate will be transferred to sterile containers, which will be kept in the dark at 1-5 °C until the arrival at the DHI laboratory, where the samples will be analysed for *E. coli* and enterococci.

For detection of *Vibrio cholerae*, one sample per replicate will be filtered through a 0.45 µm-filter, where after the filter will be placed in sterile screw-cap polyethylene tubes with approx. 10 mL of a saline-peptone solution as described in SOP 30/1707.

11.5.2 Work in laboratory

Analyses of *E. coli* and enterococci will be conducted according to SOP 30/1708. The possible occurrence of *Vibrio cholerae* will be analyzed according to SOP 30/1707.

11.6 DOC, POC and TSS

11.6.1 Work on location

For determination of dissolved organic carbon (DOC) and particulate organic carbon (POC), the samples will be treated as described in SOP 30/1769.

For determination of total suspended solids (TSS) the samples will be filtered through a glass fibre filter which has already been weighed in the laboratory.

11.6.2 Work in laboratory

Determination of DOC and POC according to SOP 30/1769. Determination of TSS according to SOP 30/1768.

12 VALIDITY CRITERIA

Valid test cycles according to the guidelines (/1/; Annex, Part 2, Section 2.2.2.5) are test cycles in which:

- The concentrations of viable organisms in the inlet water are at least 10 times higher than the maximum permitted values in regulation D-2.1 on discharge (excepted from the requirements to bacteria);

- The concentrations of viable organisms in the control discharge water exceed the maximum permitted values in regulation D-2.1 on discharge (excepted from the requirements to bacteria).

13 PASS CRITERIA

The guidelines (/1/; Annex, Part 2, Section 2.2.2) prescribe that the performance evaluation in the shipboard test may be considered successful, if the BWMS has passed the criteria below in three consecutive test cycles, including ballasting and de-ballasting operations, conducted on board a vessel during a trial period of not less than six months.

The pass criteria for the shipboard test cycles are:

1. The test cycle shall be valid according to the validity criteria
2. The average density of organisms larger than or equal to 50 µm in minimum diameter in the replicate samples shall be less than 10 viable organisms per m^3 at discharge
3. The average density of organisms smaller than 50 µm and larger than or equal to 10 µm in minimum diameter in the replicate samples shall be less than 10 viable organisms per mL at discharge
4. The average density of *Vibrio cholerae* (serotypes O1 and O139) shall be less than 1 CFU per 100 mL at discharge
5. The average density of *E. coli* in the replicate samples shall be less than 250 CFU per 100 mL at discharge
6. The average density of intestinal enterococci in the replicate samples shall be less than 100 CFU per 100 mL at discharge

14 TIME SCHEDULE

June-July 2012	First campaign of test cycle(s) conducted on board
1 August	Interim report after 1st test cycle
January 2013	Second campaign of test cycle(s) conducted on board
February-March 2013	Draft and final reporting. Final report submitted two months after completion of the second campaign.

15 QUALITY ASSURANCE AND CONTROL

The project will be conducted in accordance with the principles of ISO 9001 by using the DHI Business Management System and the procedures in the quality management plan /2/.

Quality assurance will include internal audit carried out by quality assurance personnel independent of the staff involved in the shipboard test according to procedures described in the DHI Business Management System referred to in the quality management plan /2/.

The DHI quality supervisor will approve this quality assurance project plan and conduct quality control of reports.

16 REPORTS

The following reports will be prepared:

- An interim report compiling the data for the first campaign of test cycle
- A draft final report compiling all relevant data from the three test cycles, data interpretation and conclusion

- A final report

17 ARCHIVING

All data generated and all other records and information relevant to the quality and integrity of the shipboard test will be retained according to the quality management plan /2/. The data will be filed in the archives of DHI and retained for a period of five years after issue of the final report.

18 AMENDMENTS AND DEVIATIONS

Amendments are planned changes of the quality assurance project plan. Deviations are unplanned changes. Amendments and deviations will be signed by the project manager and documented in the file and the final report.

19 REFERENCES

/1/ Resolution MEPC.174(58). Adopted on 10 October 2008. Guidelines for approval of ballast water management systems (G8).

/2/ Quality management plan. Performance evaluation of ballast water management systems. DHI Denmark. Version 3.1. June 2012.

APPROVAL OF QUALITY ASSURANCE PROJECT PLAN

DHI Ballast Water Centre

Project management Gitte I. Petersen Date: 11/6-2012

Quality control Torben Madsen Date: 11/6-2012

This quality assurance project plan is accepted and my signature authorises the study to proceed as described in this document.

Client Charlène Ceresola
BIO-UV Date: 11/06/2012

APPENDIX A

Description of the ballast water management system as given by the client

BIO SEA

General description

Summary

1. GENERAL DESCRIPTION OF BIO-SEA SYSTEM

1.1 Definition:

BIO-SEA is a system specifically designed for ballast water treatment. It consists of two treatment steps:

- ☐ Step 1: MECHANICAL FILTRATION. It aims at reducing the amount of total suspended particles, organic or not, present in the sea water.

  Step 2: ULTRAVIOLET DISINFECTION. Without any addition of chemicals nor creation of active substances, it inactivates the microorganisms present in the water (bacteria, phytoplankton, zooplankton).

When ballasting, both operations of filtration and UV disinfection are carried out: the objective is to limit the loading of suspended solids and living microorganisms in the ballast tanks.
When deballasting, only the operation of UV disinfection is completed (the filter is by-passed). This allows re-treating the water that stayed in the ship's ballast tanks during the journey, in order to eliminate the possible biological recontamination, to ensure compliance with the IMO standards for discharge of ballast water.
The entire operation of the BIO-SEA system is automated (valves opening and closing, filter cleaning, UV intensity regulation)

1.2 Data Table:

BWTS system	Flow rate in m^3/h									
	100	200	300	400	500	600	700	800	900	1000
Filter (fig1.a)										
Reactor (fig1.b)	x1	x2	x3	x4	x5	x6	x7	x8	x9	x10
2x22kW cabinet (fig1.c)			x1	x1	x2	x2	x3	x3	x4	x4
22kW cabinet + HMI (fig1.d)	x1		x1		x1		x1		x1	
2x22kW cabinet + HMI (fig1.d)		x1		x1		x1		x1		x1
Control cabinet (fig1.e)	x1	x1	x1	x1	x1	x1	x1	x1	x1	x1

Electric specifications	Power consumption									
	100	200	300	400	500	600	700	800	900	1000
Total Amperage for 380V 3~ 60Hz (A)	50	88	126	164	202	240	278	316	354	392
Total Amperage for 440V 3~ 60Hz (A)	43	76	109	142	175	207	240	273	306	339
Power of power cabinet (kW)	25	50	75	100	125	150	175	200	225	250
Power of control cabinet (kW)	8	8	8	8	8	8	8	8	8	8
Max Total Power (kW)	33	58	83	108	133	158	183	208	233	258

1.3 Description of subassembly:

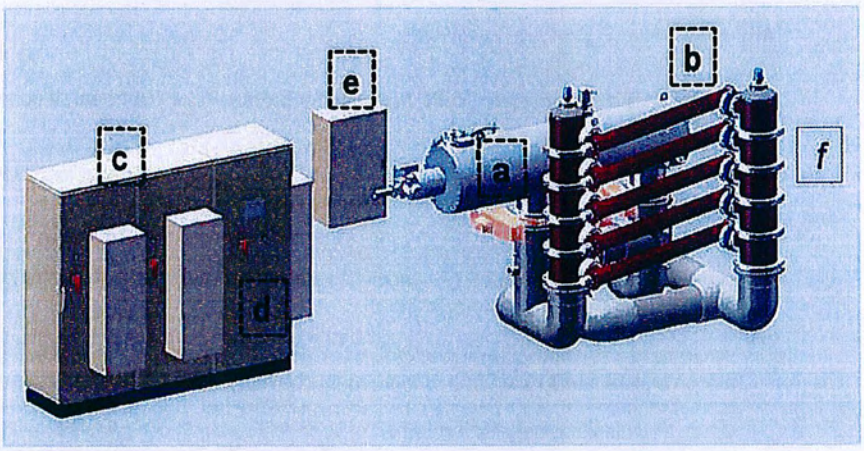


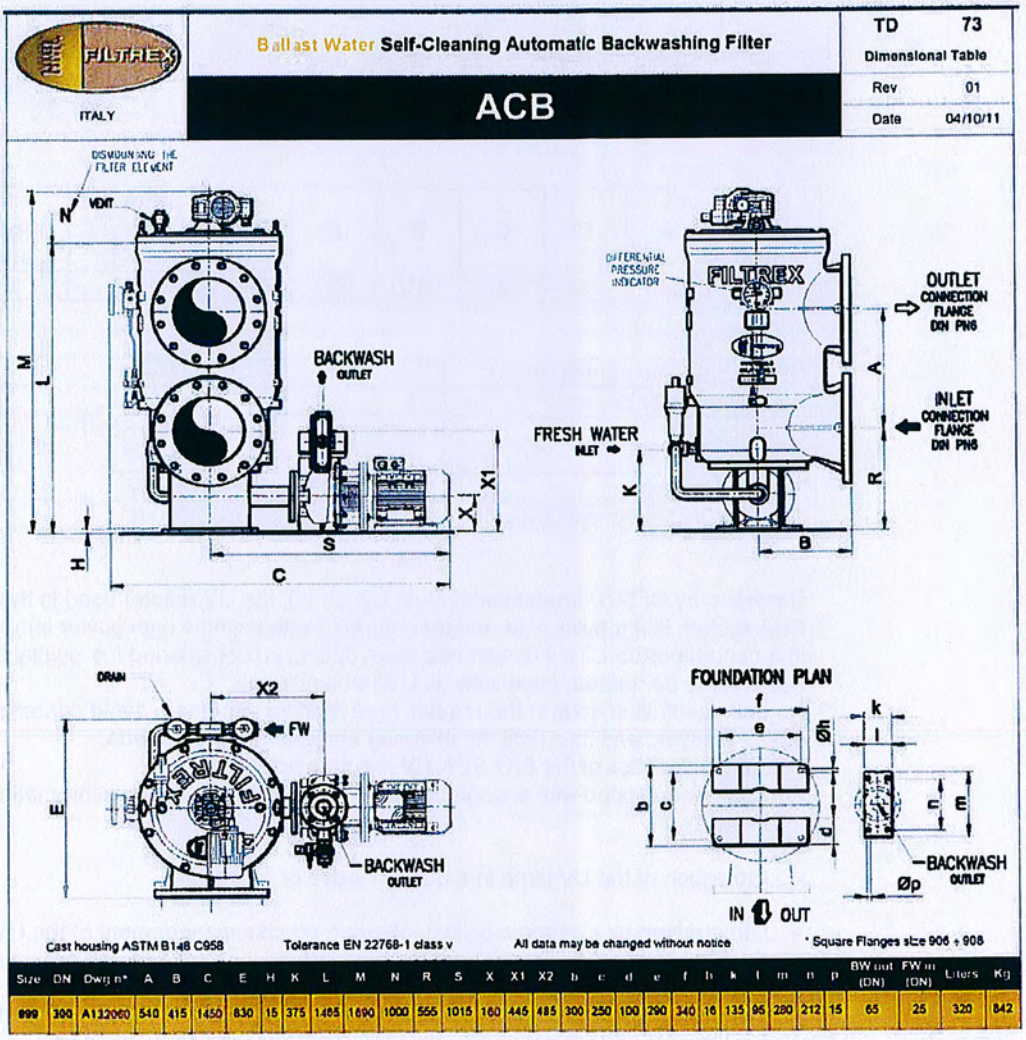
Fig.1

a. Filter

I. Description of the Filter

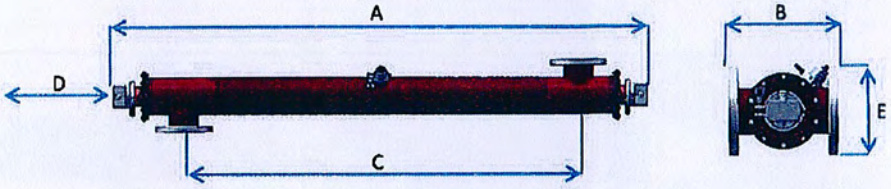
The filter presents a cylindrical housing with a filtration screen (mesh) of 40µm. An extended filtering surface allows a good retention rate. The needed surface is dimensioned depending on the flow rate to treat.
Filtration is carried out from inside (entry of dirty water) to outside (clean water).
The clogging level of the filter screen is monitored by a differential pressure switch. When the pressure differential reaches the defined threshold value, indicating filter clogging, a suction device is automatically started to clean the screen.
A specific pump boosts the suction process to unstick the clogged solids, and drove them back to the original seawater (harbor) through the discharge pipe, so that the suspended solids and biggest organisms are pumped back into the medium from which they came.
The cleaning cycle of the filter screen does not disrupt the filtration process, allowing no significant variation of the treated flow rate.
At the end of the ballasting operation, the filter is drained and refilled with fresh water.

ii. Datasheet for 1000m^3/h



Size	DN	Dwg n°	A	B	C	E	H	K	L	M	N	R	S	X	X1	X2	b	c	d	e	f	h	k	l	m	n	p	BW out (DN)	FW in (DN)	Liters	Kg
999	300	A132060	540	415	1450	830	15	375	1485	1690	1000	555	1015	160	445	485	300	250	100	290	340	16	135	95	280	212	15	65	25	320	842

b. Description of UV reactors



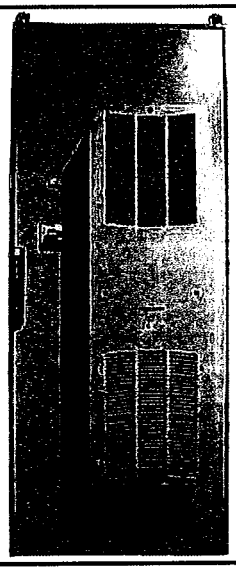
Size in mm	A	B	C	D	E	Flange	Reactor weight (Kg)	Max operating pressure
	2051	274	1508	1810	567	DN100	26	10 bars

REACTOR	
Number of lamp per reactor	1
Electrical power per lamp	22kW
Lamp type	MEDIUM PRESSURE
UV power per lamp	3 300 W
Total UV power	3 300 W
Average life time expectancy	3 000 h

Designed by CFD (Computational Fluid Dynamic), the UV reactor used in the BIO-SEA system is a tubular-type reactor equipped with a single high power lamp placed in a central position. This design has been optimized considering the quality of seawater to be treated, especially its UV transmittance.
As well, the fluid speeds in the reactor were studied in order to avoid deposits on the quartz sleeves, and thus limit the cleaning and maintenance needs.
The characteristics of the BIO-SEA UV reactors are:

- Reactor equipped with a single medium pressure UV lamp, polychromatic, high intensity.
- Protection of the UV lamp in a quartz sleeve of high purity.
- Lamp driven by electronic ballast, allowing precise management of the UV lamp in order to optimize its dimming, reduce the power consumption and prolong its life.
- Monitoring through UV sensor (intensity).
- Modular design that facilitates the installation of UV reactors in parallel, and a better adjustment to the flow that has to be treated.

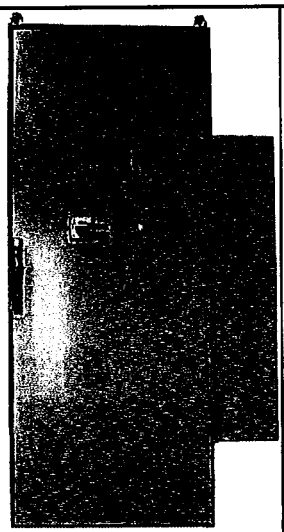
c. Description of power cabinet (without HMI)



- Equipped with 2 electronic ballasts which drive one lamp each.
- Air conditioned to preserve electronic components.
- Protected by circuit breaker for over current and by thermostat for overheating.
- Two voltage version:
  380V 3~ 50Hz or 440V 3~ 60Hz.

Size in mm	H2200xL800xP900
Weight	500kg
Material	Painted steel

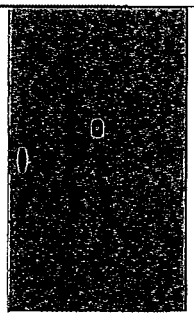
d. Description of power cabinet with HMI



- Equipped with 1 or 2 electronic depending on models.
- Air conditioned to preserve electronic components.
- Protected by circuit breaker for over current and by thermostat for overheating.
- Two voltage version:
  380V 3~ 50Hz or 440V 3~ 60Hz.
- HMI display: Monitoring of the operation of the filtration device and UV reactors, thanks to the installation of different sensors:
  • UV sensor, placed on the UV reactor: monitoring that UV intensity is above a target value and ensures the good operation of UV disinfection.
  • Temperature sensor (°C).
  • Flow meter (m3/h).
  • Differential pressure switches to control clogging cycle (bars).
- Recording of operations, alarms and measured UV intensity (10,000 records each) to cover a history of 24 months.

Size in mm	H2200xL1100xP700
Weight	500kg
Material	Painted steel

e. Description of control cabinet

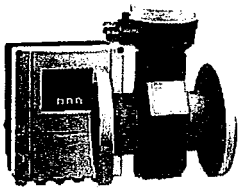


- A PLC allows to automatically operate and monitor the BIO-SEA treatment system
  The main functions are:
  - Automatic management of "ballasting" and "deballasting" modes.
  - All sensors, actuators, motors and pumps are wired directly in this cabinet.
- Voltage version:
  380V-440V 3~ 50-60Hz.

Size in mm	H1000xL650xP300
Weight	60kg
Material	Painted steel

1.4 Description of sensors:

a. Description of Flowmeter



- The Flow meter controls flow of the ballast water piping during ballasting and deballasting.
- Electromagnetic Flow meter.
- Voltage : 24Vdc.
- 4-20mA output for flow monitoring and data recording.
- Flow is displayed on the touchscreen in m^3/h.

b. Description of UV sensor

- The UV sensor controls UV Intensity during ballasting and deballasting.
- It adjusts automatically lamp power by comparing UV Intensity in real time and UV Intensity threshold defines to guarantee efficiency.
- Voltage : 24Vdc.
- 4-20mA output for UV Intensity monitoring and data recording.
- UV Intensity is displayed on the touchscreen in W/m^2.

c. Description of Temperature sensor

	- Temperature sensor controls temperature of the reactor, it stops the system if the temperature exceeds the threshold. - Temperature threshold is at 70°C - PT100 sensor. - Temperature is displayed on the touchscreen in °C.

APPENDIX B

Data of the Ship and BWT installation

SHIP SPECIFIC PARTICULARS

Name of vessel	**CMA CGM MOZART**
Ship type	**CONTAINER SHIP**
Call Sign	**FZQM**
IMO Number	**9280615**
Classification society	**BUREAU VERITAS**
Flag	**FRANCE**
Owner	**CMA CGM HEAD OFFICE**
Manager/Operator	**CMA CGM**
Year built	**2004**
Shipyard	**SAMSUNG HEAVY INDUSTRY**
Length of ship (L.O.A)	**277,30 M**
Beam	**40 M**
Nominal TEUs	**5782**

Total water ballast capacity **14289,90 M3**
Total number of segregated ballast tanks on board: **18**
Number & capacity of pump(s) **2 x 1000m^3/hour**

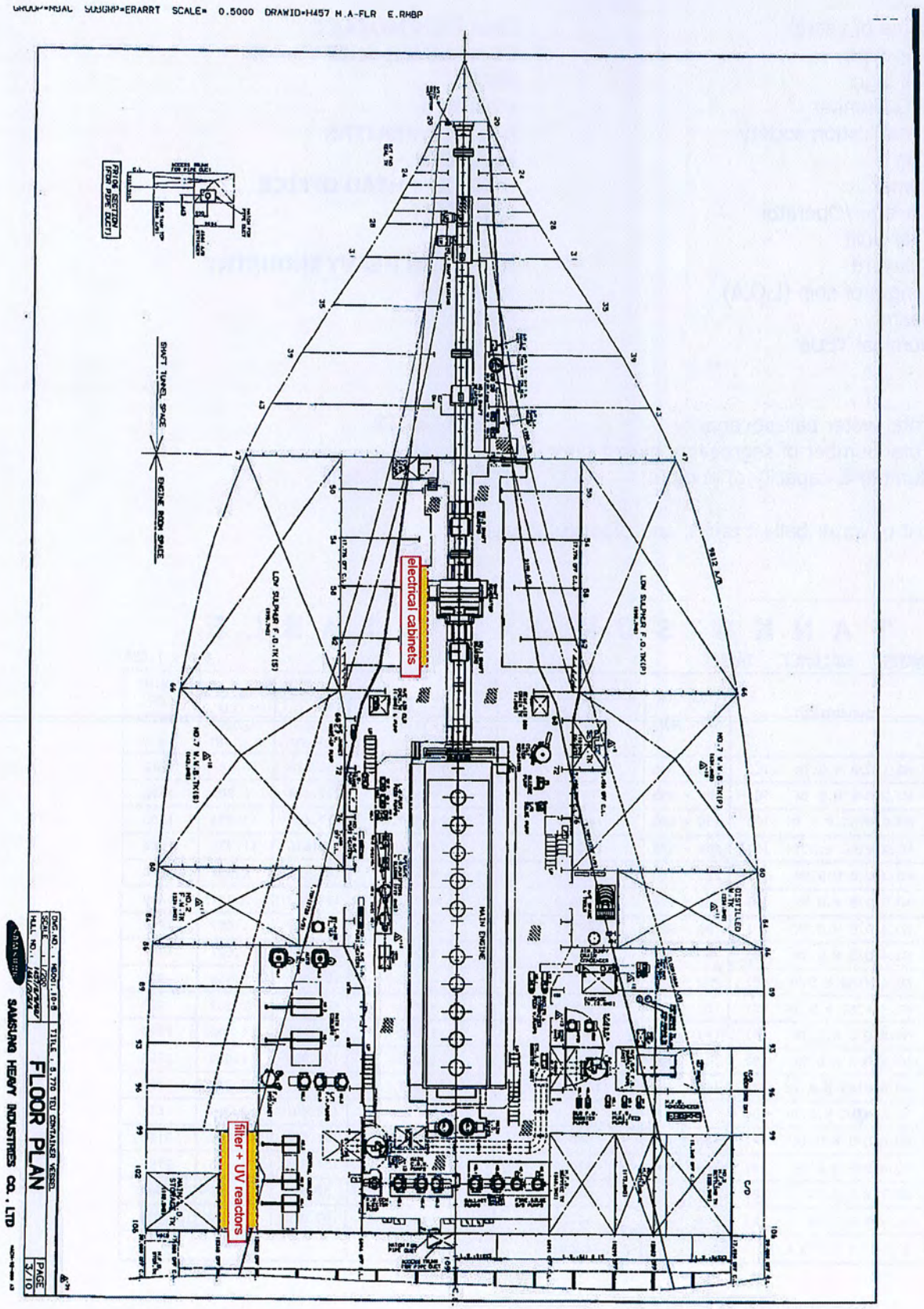
List of water ballast tanks, and capacity of each:

T A N K S S U M M A R Y T A B L E

WATER BALLAST TANKS S.G : 1.025

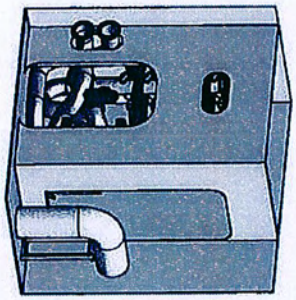
COMPARTMENT	LOCATION (FR. NO)	CAPACITIES VOLUME 100% FULL (M^3)	CAPACITIES WEIGHT 100% FULL (TONNES)	100% FULL L.C.G. FROM A.P. (M)	100% FULL V.C.G. ABOVE B.L (M)	MAX.MT OF INERTIA (M^4)
NO.1 D/B W.B.TK (C)	295 - 305	1095.6	1123.0	236.840	10.447	4242
NO.2 D/B W.B.TK (C)	258 - 295	842.8	863.8	217.468	2.805	4552
NO.2 WING W.B.TK (P)	258 - 295	1461.7	1498.3	217.646	11.774	1645
NO.2 WING W.B.TK (S)	258 - 295	1461.7	1498.3	217.646	11.774	1645
NO.3 D/B W.B.TK (P)	220 - 258	604.2	619.3	187.193	2.651	1490
NO.3 D/B W.B.TK (S)	220 - 258	604.2	619.3	187.193	2.651	1490
NO.4 D/B W.B.TK (P)	182 - 220	499.9	512.4	158.153	1.021	1897
NO.4 D/B W.B.TK (S)	182 - 220	499.9	512.4	158.153	1.021	1897
NO.4 WING W.B.TK (P)	182 - 220	463.2	474.8	159.520	2.902	925
NO.4 WING W.B.TK (S)	182 - 220	463.2	474.8	159.520	2.902	925
NO.5 D/B W.B.TK (P)	144 - 182	586.1	600.8	129.960	1.000	2525
NO.5 D/B W.B.TK (S)	144 - 182	586.1	600.8	129.960	1.000	2525
NO.5 WING W.B.TK (P)	144 - 182	1404.2	1439.3	129.801	7.329	457
NO.5 WING W.B.TK (S)	144 - 182	1404.2	1439.3	129.801	7.329	457
NO.6 D/B W.B.TK (P)	106 - 144	530.2	543.4	101.526	1.018	2195
NO.6 D/B W.B.TK (S)	106 - 144	530.2	543.4	101.526	1.018	2195
NO.7 W.W.B.TK (P)	66 - 80	619.5	635.0	57.594	9.728	1309
NO.7 W.W.B.TK (S)	66 - 80	633.0	648.8	57.540	9.669	1309
SUB TOTAL		14289.9	14647.2			

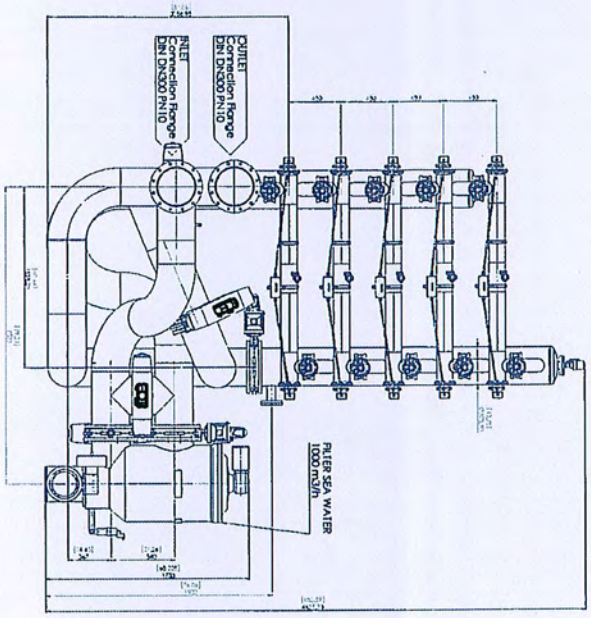
LOCATION OF THE BIO-SEA SYSTEM ONBOARD

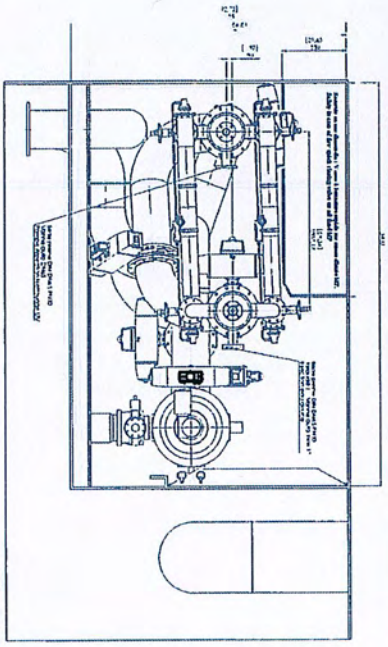


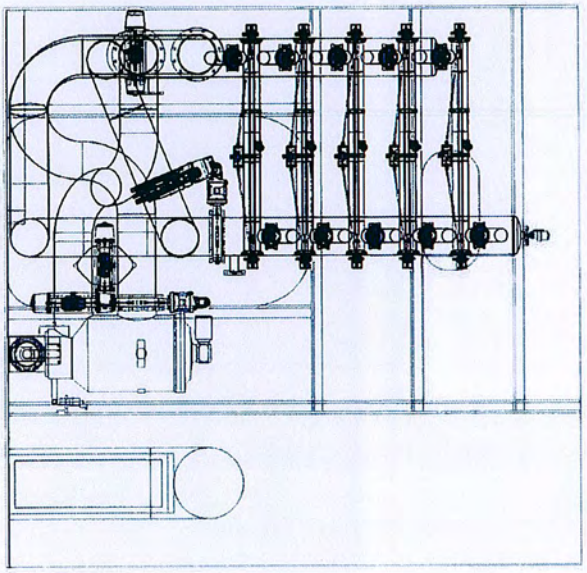
ONBOARD CONFIGURATION :

1 SYSTEM BIO-SEA 1000 M3/H (FILTREX FILTER, 10 UV REACTORS)









AMENDMENT

QAPP

Performance evaluation in shipboard test of the BIO-UV ballast water management system BIO-SEA.

Amendment number 1

26.11.2012

Amendment Comments

Analyses (Organism size class ≥10 µm and < 50 µm: The test parameter '*Primary production*' has been removed from the applied analysis and replaced by the viable stain technique 'CMFDA/FDA staining'. The methodology is described in SOP 30/1701.

Reason for Amendment

Implementation of the analysis parameter *Primary production* require the use of radiolabelled substances (C^{14}). Due to security reasons related to shipping of radiolabelled compounds the analysis parameter will not any longer be used during shipboard testing.

Impact of Amendment

None.

Preventive Action

The viable stain technique 'CMFDA/FDA staining' will be used for determining the number of viable organisms in the size range ≥10 µm and < 50 µm in the control and treated discharge water. After sampling on board the ship the samples will be shipped to DHI for further analysis.

Gitte I. Petersen
Project manager

Date: 26.11.2012

Copy to be sent to the client, the Certification Body and the DHI Quality Assurance Unit.

APPENDIX D

Certificate of compliance, ISO 9001 certificate, accreditation and GLP authorisation

DNV BUSINESS ASSURANCE

MANAGEMENT SYSTEM CERTIFICATE

Certificate No. 109333-2012-AQ-DEN-DANAK

This is to certify that

DHI Group

has been found to conform to the management system standard:

DS/EN ISO 9001:2008

This certificate is valid for the following product or service ranges:

Consulting, software, research & development and laboratory testing, analysis & products within the area of water, environment & health

Locations included in the certification will appear in the appendix.

Place and date:

Hellerup, 2012-03-23

DET NORSKE VERITAS,
BUSINESS ASSURANCE, DANMARK A/S

This certificate is valid until:

2015-01-10

DANAK
SYSTEM Reg.nr. 5001

The audit has been performed under the supervision of:

Jan Carsten Schmidt
Lead Auditor

Jens Peter Høiseth
Managing Director

Lack of fulfilment of conditions as set out in the Certification Agreement may render this certificate invalid.

DNV BUSINESS ASSURANCE

APPENDIX TO CERTIFICATE

This appendix refers to certificate no. 109333-2012-AQ-DEN-DANAK

DHI Group

Locations included in the certification are as follows:

Site Address	Scope:
Agern Allé 5 2970 Hørsholm, Denmark	Consulting, MIKE© by DHI Software Development, Sales & Support, Solutions Software Development, Research, Development & Innovation and Laboratory Analysis, Testing & Products
INCUBA Science Park**,** Gustav Wieds Vej 10 8000 Århus, Denmark	Consulting, Solutions Software Development and Research, Development & Innovation
Drakegatan 6, 412 50 Göteborg, Sweden	Consulting, MIKE© by DHI Software Sales & Support
Kyrkogatan 3, 222 22 Lund, Sweden	Consulting, MIKE© by DHI Software Sales & Support
Svartmangatan 18, 111 29 Stockholm, Sweden	Consulting, MIKE© by DHI Software Sales & Support
Honnörsgatan 16, Box 3287, 350 53 Växjö, Sweden	Consulting, MIKE© by DHI Software Sales & Support

This certificate is valid until:

2015-01-10

The audit has been performed under the supervision of:

Jan Carsten Schmidt
Lead Auditor

DANAK
SYSTEM Reg.nr. 5001

Place and date:

Hellerup, 2012-03-23

DET NORSKE VERITAS,
BUSINESS ASSURANCE, DANMARK A/S

Jens Peter Høiseth
Managing Director

Lack of fulfilment of conditions as set out in the Certification Agreement may render this certificate invalid.

COPY

Certificate no: **DS/I093222-A**
Page 1 of 1

Certificate of Compliance

Office: **Lloyd's Register EMEA**
Copenhagen Design Support Centre, Statutory Section
Strandvejen 104A, 2nd floor
DK-2900 Hellerup
Denmark

Date: **09 May 2012**

This certificate is issued to **DHI Ballast Water Centre, Denmark**

DHI Ballast Water Centre, Denmark

The Document(s) listed in paragraph 1 of the appendix have been examined for compliance with:

- Resolution MEPC.174(58), Annex part 2

and are found to comply from quality assurance and quality control aspects subject to the following:

1.1. It is required to maintain full and accurate log files in order to demonstrate correct quality measures

1.2. The Quality Assurance Project Plan is a project specific document and should as such be subject to review and commenting prior to each project start-up.

1.3. This design appraisal document is to be kept together with quality management plan.

1.4. Subject certificate is valid until 15 June 2015.

1. The documents listed below have been examined

Drawing No.	Rev.	Title	Status	Date
Date: 07 Sep 2011	**2.3**	**Quality Management Plan**	**B**	**09 May 2012**

2. The documents listed below have been considered together with the submitted documents in the appraisal

Drawing No.	Rev.	Title
11810704	**02**	**Quality Assurance Project Plan**

Appraisal Status Key

B Examined and found to comply with §2.2, Part 2 of the annex of IMO Resolution MEPC 174 (58)

Martin Schabert
Statutory Department
Copenhagen Design Support Centre
Surveyor to Lloyd's Register EMEA

LLOYD'S REGISTER EMEA
Lloyd's Register
M. Schabert
COPENHAGEN DESIGN SUPPORT CENTRE

A member of the Lloyd's Register Group

Accreditation to testing

DANAK

Company: **DHI**

Agern Allé 5
DK-2970 Hørsholm

Registration number: **26**

Valid: **24-10-2012 to 31-07-2015**

Scope:

Testing

Product

- Biological items
- Chemicals and chemical products
- Construction products
- Environmental samples

Test Type

- Biological and biochemical testing
- Chemical testing
- Microbiological testing
- Ionising radiation and radiochemistry
- Sampling

Testing is performed according to the current list of test methods approved by DANAK.

The company complies with the criteria in EN ISO/IEC 17025:2005 – General requirements for the competence of testing and calibration laboratories and demonstrates technical competence for the defined scope and the operation of a quality management system (refer joint ISO-ILAC-IAF Communiqué dated January 2009, www.danak.dk).

Issued the 24 October 2012

Jesper Høy / **Kirsten Jebjerg Andersen**

In case of any disputes, the Document in Danish language shall have priority.

COPY

DANAK

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

Laboratory inspection and study audits for compliance with the OECD Principles for Good Laboratory Practice were carried out at

Laboratory: DHI

on

Dates: 21st and 22nd October 2011

The laboratory inspection and study audits have been carried out in accordance with the regulation settled in Order No. 906 of 14th September 2009 from the Danish Ministry of Environment. The laboratory has been monitored for GLP Compliance within the following scope:

Type of products:

- *Industrial chemicals*
- *Pesticides*
- *Biocides*

Type of tests:

- *Environmental toxicity studies on aquatic and terrestrial organisms.*
- *Studies of behaviour in water, soil and air, bioaccumulation*

The laboratory was found to be operating in compliance with the OECD Principles of Good Laboratory Practice.

Date: 08 August 2012

Jesper Høy
Managing director, DANAK

Kirsten Jebjerg Andersen
GLP inspector, DANAK